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The cave insects with sex-reversed genitalia had their most recent common ancestor in West Gondwana (Psocodea: Prionoglarididae: Speleketorinae)

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Abstract

The divergence date and ancestral distributional area of the psocid subfamily Speleketorinae, which includes taxa with reversed genitalia (female penis and male vagina of *Afrotrogla* and *Neotrogla*, tribe Sensitibillini), were estimated. The most basal divergence of the subfamily (between the North American *Speleketor* and the tribe Sensitibillini) was estimated to have occurred according to the separation between the North American continent and Gondwana, ca. 175 MYA. The most basal divergence of Sensitibillini (between African *Afrotrogla* + *Sensitibilla* and Brazilian *Neotrogla*) was estimated to have occurred according to the split of West Gondwana (separation between the African and South American continents), ca. 127 MYA. The biome of the ancestral distributional area of Sensitibillini (inland of West Gondwana) is believed to be arid to semi-arid, which might strengthen the reversed sexual selection and then facilitate the origin of pre-adaptive features related to the evolution of a female penis. All extant Sensitibillini species inhabit carbonatic caves, but geological evidence suggested independent shifts of these genera to the carbonatic cave habitat in Tertiary/Quaternary.

Key words: *Afrotrogla*, female penis, *Neotrogla*, Pangea, reversed sexual selection, *Sensitibilla*, vicariance.

/Maintext

The subfamily Speleketorinae is a small group of cave dwelling psocids containing four genera, *Speleketor* (tribe Speleketorini) from North America, *Afrotrogla* and *Sensitibilla* from Africa, and *Neotrogla* from South America (tribe Sensitibillini). Some members of the subfamily are especially famous for having sex-reversed genital organs (i.e., females possess an intromittent organ, or female penis, observed in *Afrotrogla* and *Neotrogla*) (Lienhard 2007; Lienhard *et al.* 2010a; Yoshizawa *et al*. 2014, 2018a), whereas *Sensitibilla* has a less-developed pre-penis and *Speleketor* has conventional genital organs (Lienhard, 2000; Lienhard *et al.* 2010b). The female penis is protrudable and inserted into the male vagina-like cavity during copulation to receive nutritious semen from the male, and reversed direction of sexual selection caused by female-female competition for the nutritious semen is considered to be the major factor facilitating the evolution of the female penis (Yoshizawa *et al*. 2014, 2018b). The recent molecular phylogeny of Spekeletorinae showed that *Afrotrogla* and *Sensitibilla* formed a clade, and *Neotrogla* was placed to its sister taxon. Given this phylogenetic relationship and considerable differences of their female penis structures, the protrudable female penis is considered to have evolved independently in *Afrotrogla* and *Neotrogla*, respectively (Yoshizawa *et al*. 2018a). In addition to this unique sexual trait, the widely disjunct distribution of this subfamily (North American *Speleketor*, African *Afrotrogla* and *Sensitibilla* and South American *Neotrogla*) is of special interest as well because this distributional pattern seems to be formed by the continental separations between North America and Gondwana (Speleketorini – Sensitibillini) and Africa and South America (*Afrotrogla*+*Sensitibilla* – *Neotrogla*). Elucidating the environment of their ancestral distributional areas will also greatly contribute to understand the potential environmental factors which affected the origin of the female penis (Yoshizawa *et al*. 2014, 2018a,b, 2019). In this study, we estimate the biogeographical history of the Speleketorinae based on the estimations of their divergence dates and ancestral distributional areas.

We used the recently estimated molecular phylogenetic tree of the subfamily (Yoshizawa *et al*. 2018a,c) for the divergence date and ancestral area estimations. For the dating analysis, we used a Bayesian method implemented in the software MCMCtree of the PAML 4.8 software package (Yang 2007). First, we estimated the substitution rate prior using 328 MYA for the deepest divergence date of Psocodea (Trogiomorpha and Troctomorpha+Psocomorpha) according to Johnson *et al.* (2018) (A in Fig. 1). Based on the result, a gamma prior for the substitution rate was set. The GTR+G model was adopted with an alpha = 0.5, which was a close approximation of the best fit substitution model estimated by jModeltest (Posada 2008; Yoshizawa *et al*. 2018a). We performed a run for 100 000 generations, and the values were sampled every 50 generations. The first 50% of the obtained values were excluded for burn-in. Because almost no convincing fossil

evidence applicable to the present tree was available, the following six calibration points were selected according to the 95% confidence interval of divergence dates estimated by Johnson *et al*. (2018): 292.4–375.7 MYA for the deepest divergence of Psocodea (A in Fig. 1); 92.1–233.8 MYA for *Cerobasis – Echmepteryx+Soa* (B); 270.3–348.9 MYA for Troctomorpha – Psocomorpha (C); 171.0–225.2 MYA for Caeciliusetae+Homilopsocidea – Philotarsetae+Epipsocetae+Psocetae (D); 107.8–152.7 MYA for Hemipsocidae – Psocidae; and 105.6–175.3 MYA for Caeciliusetae – Homilopsocidea (F). For the ancestral area estimation, only the subfamily Speleketorinae was subjected, and the Statistical Dispersal-Vicariance Analysis (S-DIVA: Ronquist 1997; Yu *et al.* 2010) as implemented in RASP 3.2 software (Yu *et al.* 2015) was adopted with default setting. Three geographical regions were defined as follow: Africa (*Afrotrogla* and *Sensitibilla*), South America (*Neotrogla*) and North America (*Speleketor*).

Figure 1 summarizes the results from the dating and ancestral area analyses (see electronic Supporting Information for details: Appendix S1–2). The ancestral distributional area of Speleketorinae (Sensitibillini plus *Speleketor*) was estimated as North America plus South America plus Africa. The age of the most basal divergence of the subfamily was estimated to be 175.5 MYA (95% confidence interval: 103.2–265.3 MYA), which corresponded pretty well to the opening date of the Central Atlantic (i.e., separation of North American continent from Gondwana: Seton *et al.* 2012). The distributional area of the common ancestor of Sensitibillini (*Afrotrogla*, *Sensitibilla* and *Neotrogla*) was estimated to be South America plus Africa. The age of the most basal divergence of the tribe was estimated to be 127.2 MYA (95% confidence interval: 68.7–200.8 MYA), which is slightly older than the opening date of the South Atlantic Ocean (i.e., separation of African and South American continents: Nishihara *et al.* 2009; Seton *et al*. 2012). Both the dating and ancestral area analyses suggested that the common ancestor of Speleketorinae originated in the Pangea Supercontinent, and the divergence between *Speleketor* and Sensitibillini occurred according to the separation of the North American and Gondwana continents; the common ancestor of Sensitibillini were distributed in West Gondwana, and the divergence between *Afrotrogla* + *Sensitibilla* and *Neotrogla* occurred according to the separation of the African and South American continents. Thus, the current distributional pattern of Speleketorinae can convincingly be explained by vicariance events. In *Neotrogla*, significant genetic differentiation was detected even between conspecific populations from very closely located caves (1 km: Kamimura *et al.* 2019). This suggests a low level of migration in the species of Speleketorinae, although well developed eyes and wings suggest that they retain some ability to migrate.

The biogeographical history of Speleketorinae also provides some insights for the morphological and behavioral evolution of Sensitibillini. Figure 2 shows the present-day distributional areas of Speleketorinae plotted on the map of Pangea Supercontinent. The

distributional areas of *Afrotrogla* + *Sensitibilla* and *Neotrogla* are situated very close on the supercontinent, and the biome of the area during Jurassic to early Cretaceous period (Fig. 1) is thought to be an arid to semi-arid zone (Rees *et al.* 2002; Vajda & Wigforss-Lange 2009; Hay & Floegel 2012). Dry and oligotrophic cave environment of the present-day habitat of Sensitibillini is considered to be one of the important factors that strengthened the female-female competition for nutritious semen and then facilitated the origin of a female penis (Yoshizawa *et al*. 2014, 2018ab, 2019). Many preadaptive features related to the origins of the female penis (e.g., female's pre-penis and dual seminal slots for receiving double amount of semen) are considered to have originated in the common ancestor of Sensitibillini (Yoshizawa *et al.* 2018a,b). These suggest that the dry and oligotrophic paleoenvironment inhabited by the common ancestor of Sensitibillini (not necessary caves, as discussed below) might also have facilitated the origin of such preadaptive features.

All known extant Sensitibillini genera exclusively inhabit carbonatic (limestone or dolomite) caves, so that the parsimonious interpretation for their ancestral habitat would be such type of caves. Although the carbonatic layer where the caves inhabited by Sensitibillini occur is estimated to be formed during late Proterozoic (ca. 650–920 MYA) (Irish *et al.* 2001; Auler *et al*. 2001), most caves associated to the limestone area are thought to be formed in the late Tertiary or in Quaternary, although some hypogenic caves can present much older origins (as the Cambrian period: Klimchouk *et al*. 2016). However, even in those cases of ancient hypogenic speleogenesis, dating from early Paleozoic (as the Toca da Boa Vista cave, where *Neotrogla truncata* can be found), the colonization of the caves certainly occurred much later (in late Tertiary or Quaternary), since only in those periods the caves were “opened” to the surface, thus allowing external lineages to colonize them. Therefore, it is very unlikely that the common ancestor of Sensitibillini inhabited carbonatic caves. Many species of the close relatives of Sensitibillini (Fig. 1), i.e., Prionoglaridinae, *Speleketor*, and Psyllipsocidae (from which *Dorypteryx* was sampled here: Fig. 1), are generally recognized as cave dwellers as well, but their connection with caves is not as tight as in Sensitibillini. For example, some *Prionoglaris* and *Psyllipsocus* have also been collected from small spaces or hollows of rocks (Lienhard 1988; Lienhard & Garcia Aldrete 2016), and *Speleketor irwini* is found on the skirts of dead leaves of the desert fan palm (Mockford 1993). Probably, the common ancestor of Sensitibillini also inhabited such environments and then the African and South American Sensitibillini changed their habitat to the carbonatic caves independently. This relatively recent change to the cave environment may also explain the preservation of well-developed eyes and flight ability (Ogawa & Yoshizawa 2018) in Sensitibillini. The female penis is thought to have evolved independently in *Afrotrogla* and *Neotrogla* (Yoshizawa *et al.* 2018a), which may also be related to their independent habitat shifts to the dry and oligotrophic caves (the type locality cave of *Sensitibilla strinatii* is dry but is colonized by bats that produce lots of guano, a copious food

resource for these psocids: RLF personal observation 2018).

All genera and species of Sensitibillini were recently described, and it is very likely that unknown genera and species are still to be discovered. Future alpha-taxonomic studies may lead to further insight into the morphological and behavioral evolution of this group.

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SUPPORTING INFORMATION

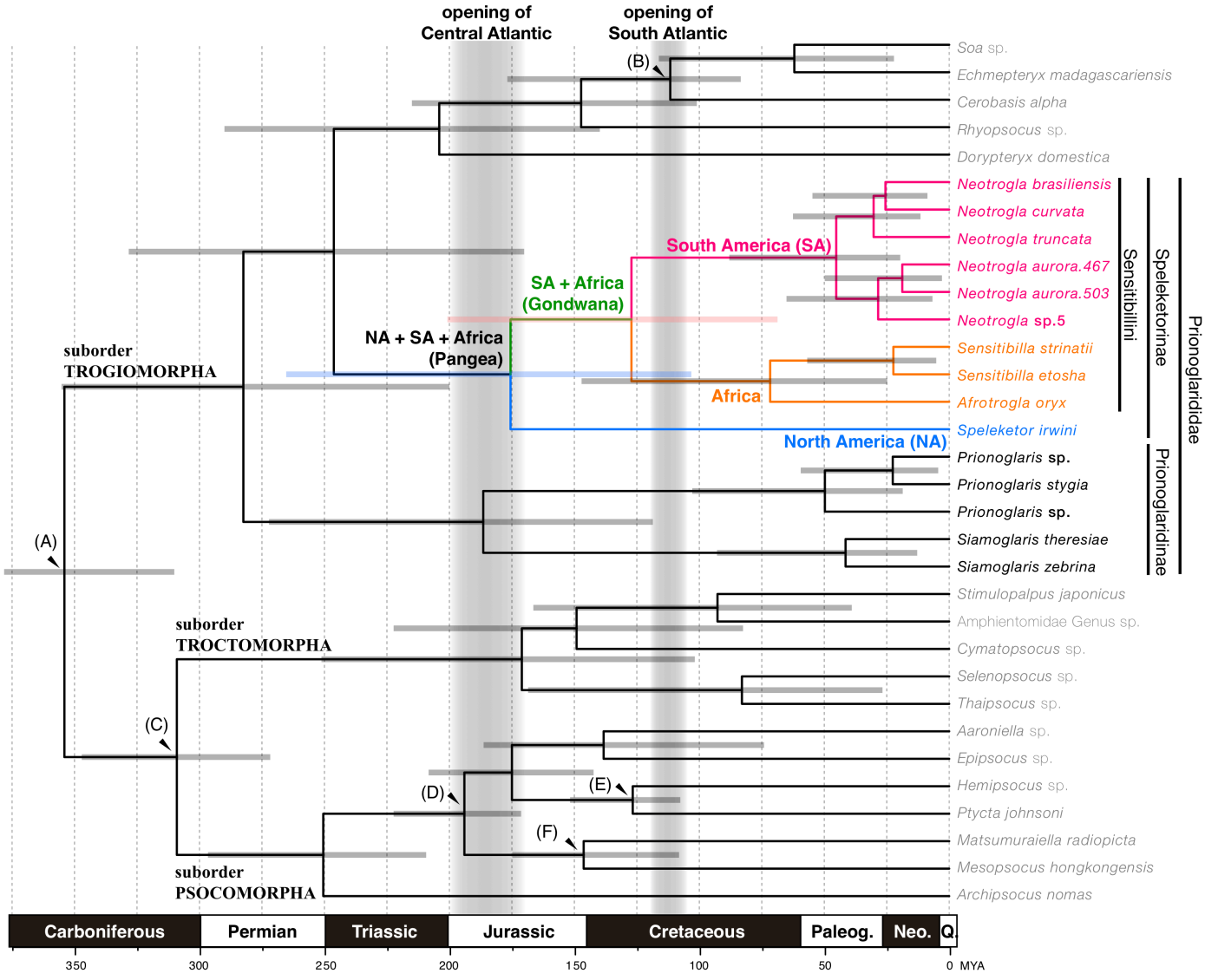
Appendix S1. Newick formatted tree file containing the divergence dates estimated by MCMCtree.

Appendix S2. Text file containing the detailed results from the S-DIVA analysis.

Figure captions

Figure 1 Chronogram of Speleketorinae estimated by Bayesian Markov chain Monte Carlo method. Generally accepted dates of continental separations are indicated by vertical bands (band width indicates range of estimated dates from different literatures). The ancestral distributional area estimated by S-DIVA are noted along the branches. (A)–(F) indicate calibration points used for the dating analysis (see text for details). 95% confidence intervals are indicated by horizontal bars.

Figure 2 Present-day distributional areas of the speleketorine genera plotted on the map of Pangea with modern continental outlines (map modified from Wikipedia). Arid to Semi-Arid paleoclimate zone is indicated according to Rees *et al.* (2002).


opening of Central Atlantic
opening of South Atlantic
(B)
Soa sp.
Echmepteryx madagascariensis
Cerobasis alpha
Rhyopsocus sp.
Dorypteryx domestica
Neotrogla brasiliensis
Neotrogla curvata
Neotrogla truncata
South America (SA)
Neotrogla aurora.467
Neotrogla aurora.503
SA + Africa (Gondwana)
Neotrogla sp.5
NA + SA + Africa (Pangea)
Sensitibilla strinatii
Sensitibilla etosha
Africa
Afrotrogla oryx
Speleketor irwini
North America (NA)
Prionoglaris sp.
Prionoglaris stygia
Prionoglaris sp.
Siamoglaris theresiae
Siamoglaris zebrina
suborder TROGIOMORPHA
(A)
Stimulopalpus japonicus
Amphientomidae Genus sp.
suborder TROCTOMORPHA
Cymatopsocus sp.
Selenopsocus sp.
Thaipsocus sp.
(C)
Aaroniella sp.
Epipsocus sp.
(D)
(E)
Hemipsocus sp.
Ptycta johnsoni
(F)
Matsumuraiella radiopicta
Mesopsocus hongkongensis
suborder PSOCOMORPHA
Archipsocus nomas
Sensitibillini
Speleketorinae
Prionoglaridinae
Prionoglaridae
Carboniferous
Permian
Triassic
Jurassic
Cretaceous
Paleog.
Neo.
Q
350
300
250
200
150
100
50
0 MYA

60º
Eurasia
30º
North America
Arid to Semi-Arid
Speleketor
0º
Africa
South America
30º
Afrotrogla
+
Sensitibilla
Neotrogla
India
60º
Antarctica
Australia