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Singing in the Namoroka Caves, First Record In Situ for a Cave Dwelling Insect: *Typhlobrixia namorokensis* (Hemiptera, Fulgoromorpha, Cixiidae)

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Abstract This is the first substrate-borne communication record of the troglobitic planthopper *Typhlobrixia namorokensis* in the caves of the Ambovonomby network, Namoroka Tsingy, Madagascar. A supplementary morphological description of the male genitalia is provided and, for the first time, female genitalia of *T. namorokensis* are characterized. Molecular data were obtained for *T. namorokensis*; molecular analysis also reveals the presence of another (yet unidentified) cavernicolous cixiid in the Antsifotra caves network. The evolutionary origin of *T. namorokensis* is discussed. Its adaptation to caves and its behaviour allows its identification as a true troglobiont. Its vibrational signal structure is compared to the calls of other cave-dwelling cixiids.

Keywords Substrate-borne communication · troglomorphy · cave adaptations · Auchenorrhyncha · call structures

Introduction

Substrate-borne communication has been known for a long time for many groups within the Hemiptera (Ossiannilsson 1949; Claridge 1985; Čokl and Virant-Doberlet 2003), an attribute that should be considered ancestral for the group

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(Soulier-Perkins et al. 2007) even if we are far from having documented such behaviour in all the families of Hemiptera. The use of vibrational signals can be related to alarm and defensive behaviour but it is essential in order to recognise and locate conspecific partners for reproduction (Virant-Doberlet and Čokl 2004). In addition to vibratory signal diversity, mate searching strategies using such signals can be very different from one species to another (de Groot et al. 2011). Presently, around 30 families in this order are known to produce vibrational signals, and several species have been recorded from 11 different families of Fulgoromorpha (Table 1).

Within the planthoppers, independent evolutionary lineages have colonized caves and are part of communities which feed on roots of the epigean vegetation that penetrate deeply into the caves. Approximately 50 cave-dwelling species are reported from many parts of the world, as summarised in Hoch and Wessel (2006).

T. namorokensis is described from the Tsingy of Namoroka, a characteristic Malagasian karstic area located in the National Park of Namoroka. It is a strict nature reserve of approximately 220 km^2 (Figs. 1, 2) located in the northwestern part of the island of Madagascar, Mahajanga Province, about 50 km south of Soalala city. The species was previously known only from two adult males (holotype and paratype) and two nymphs. In this paper, we provide new details of the male genitalia and describe for the first time the female. We also present the first photographs of the species in its natural environment, and we report and analyse the vibratory communication signals of *T. namorokensis*. While intraspecific communication by substrate-borne vibrations has been demonstrated in other obligatory cavernicolous planthoppers from Hawaii and Australia (Hoch and Howarth 1993; Hoch 2000; Wessel et al. 2013) and in an endogean species from Germany (Hoch et al. 2013), this is the first in situ signal recording of a troglobitic planthopper. We also describe the habitat of *T. namorokensis* from its type locality, the caves of Namoroka, and present information on *T. namorokensis* biology.

Table 1 Fulgoromorpha families producing vibrational signal

Family	Reference
Acanaloniidae	Wilson MR unpublished data
Caliscelidae	Tishechkin 2006
Cixiidae	Ossiannilsson 1949; Tishechkin 1997
Delphacidae	Strübing 1958; Claridge 1985
Derbidae	Tishechkin 2006; 2008
Dictyopharidae	Strübing 1977; Tishechkin 1997
Flatidae	Moore 1961; Virant-Doberlet and Čokl 2004
Fulgoridae	Gogala M, Soulier-Perkins A both unpublished data
Issidae	Tishechkin 1997, 1998
Lophopidae	Soulier-Perkins et al. 2007
Tropiduchidae	Tishechkin 1997

Figs. 1–2 Madagascar map, *1*) Location of the Namoroka park in comparison to the coast and location of caves network explored; *2*) Location of the park within Madagascar

Material and Methods

Morphological Descriptions

T. namorokensis specimens were studied and illustrated using a Leica MZ12 stereomicroscope with *camera lucida* attachment. Habitus photographs were taken with a Canon EOS 350D digital, 65 mm f2.8 lens and Canon Macro Twin Lite MT-24EX flash. Morphological terminology follows that of Bourgoin and Huang (1990) and Bourgoin (1993) for male and female genitalia respectively, and Bourgoin et al. (2015) for forewing vein nomenclature. All specimens were collected by visual search in the caves network of the Namoroka National park in the Mahajanga Province of Madagascar in September 2012 and are deposited in the Muséum national d'Histoire naturelle, Paris, France (MNHN) and the Natural History Museum, London, UK.

Molecular Extraction, Amplification and Sequencing

Whole genomic DNA was extracted from muscle tissue with a DNeasy Tissue kit (Qiagen) from 5 individuals, 3 nymphs and 2 adults. Two of the nymphs (MNHN (EH) 19272 and 19263) and the 2 adults (MNHN (EH) 19262 and 19264) were collected in the caves belonging to the Ambovonomby network. The third nymph (MNHN (EH) 19265) was collected in caves (NA27 and NA40) belonging to another network, Antsifotra, separated from the Ambovonomby network by 12 km.

Portions of the 18S rDNA was amplified with the following primers: 574 (GCCGCGGTAATTCCAGCT), E21 (CTCCACCAACTAAGAACGG), 18S-mid (GATACCGCCCTAGTTCTAACC) and 2200 (CGGCAGGTTCACCTACGG) (Bourgoin et al. 1997). CO1 was amplified using a mix of different primers Hemiptera specific: LCO1490 puc_t1 et LCO1490 hem1_t1 (forward) and HCO2198 puc_t1, HCO2198 hem1_t1 et HCO2198 hem2_t1 (reverse) (CBGP, INRA Montpellier).

The resulting sequences have been assembled using CodonCode aligner version 5.1.4 (CodonCode Corporation 2014) and deposited in GenBank under the registration numbers KT602430, KT602431, KT602432 and KT602433.

Signal Recording and Analysis

T. namorokensis males and females were collected with some roots on which they were found feeding. The insects along with the roots were placed in a chamber, a transparent plastic box (18×14×6 cm) where substrate-borne vibrations were recorded using a BSR ST8 diamond stylus LP/78 magnetic cartridge. The roots were fixed at the bottom of the box with some UHU Patafix and blocked at the top with the lid. The cartridge/stylus was fixed on a wooden stick 12 cm long and 1.5 cm in diameter. This stick could be moved through a hole on the side of the chamber, allowing us to move the stylus sideways and to place the diamond of the stylus in contact with the roots. The magnetic cartridge was plugged into a small 9 V amplifier (amplifying the signal by a factor of 50) connected to an Edirol Roland R-09HR recorder (Fig. 3). Recordings were taken in the cave where the insects and their host-plants were found. The ambient temperature was 19.9–20.4 °C with a relative humidity of 73–83 %. Four males and two females were present in the chamber when the signal was recorded.

The signal was recorded at a sampling frequency of 44.1 kHz and 32-bit resolution. Then it was downsampled to 5 kHz to increase the frequency resolution (The frequency resolution of a 44.1khz signal with a 512-sample FFT is 86.13 Hz; downsampling to 5Khz increases frequency resolution to 9.77 Hz).

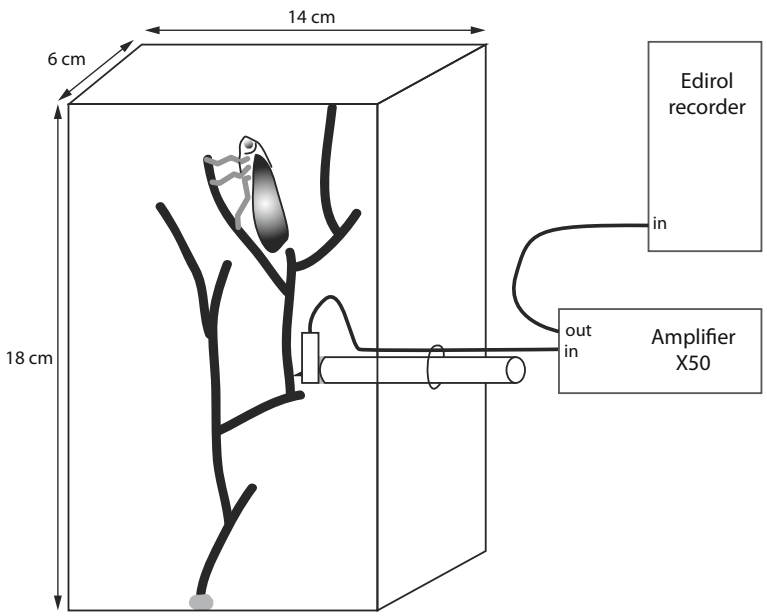


Fig. 3 Recording setup

The calls were analysed using Audacity 2.0.2 (Audacity 2012) and seewave software packages, with a FFT of 512 samples (Sueur et al. 2008). The samples used for the analysis are registered in the MNHN sound library under the registration numbers MNHN SO 2015-7 and MNHN SO 2015-15.

Results

T. namorokensis Habitat

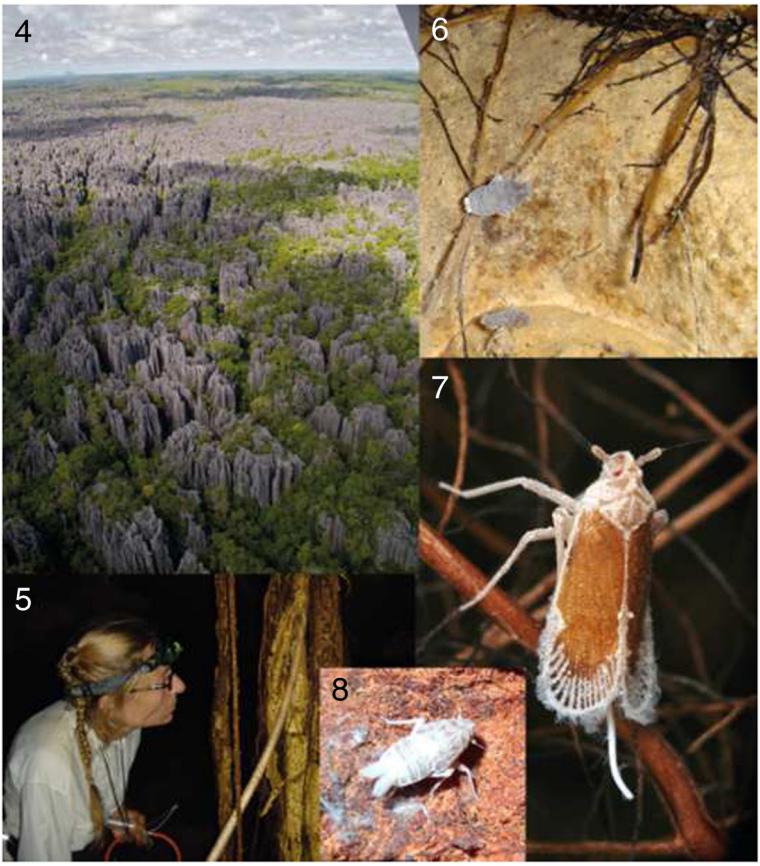
Tsingy of Namoroka is a unique and spectacular landscape composed of a calcareous plateau cut into sharp structures of limestone walls and canyons where natural swimming pools and caves are carved out (Fig. 4). These structures are known in Malagasy as Tsingy. This very remote area is difficult to access, and can only be explored during the dry season that generally lasts 7 months of the year from April to October. During the rainy season, the lower parts of the karst are completely flooded and crocodiles are present. Caves and galleries are numerous and interconnect throughout the endokarst, resulting in a large and complex network. Clarke (2003) provided detailed descriptions of the karst biospace of Madagascar.

A very open, patchy deciduous forest grows on the upper parts of the massif, and the roots of the trees reach into the caves. They can hang from the ceiling and may grow to a size that build large columns reaching the floor (Fig. 5) or develop into a fine network along all the walls, the floor and any fissures and cracks in the caves (Figs. 6 and 7). *Typhlobrixia* adults were always found closely associated with those fine roots, standing on them while single nymphs were simultaneously observed on the walls, sometimes far from any roots.

Supplementary Description of *T. namorokensis*

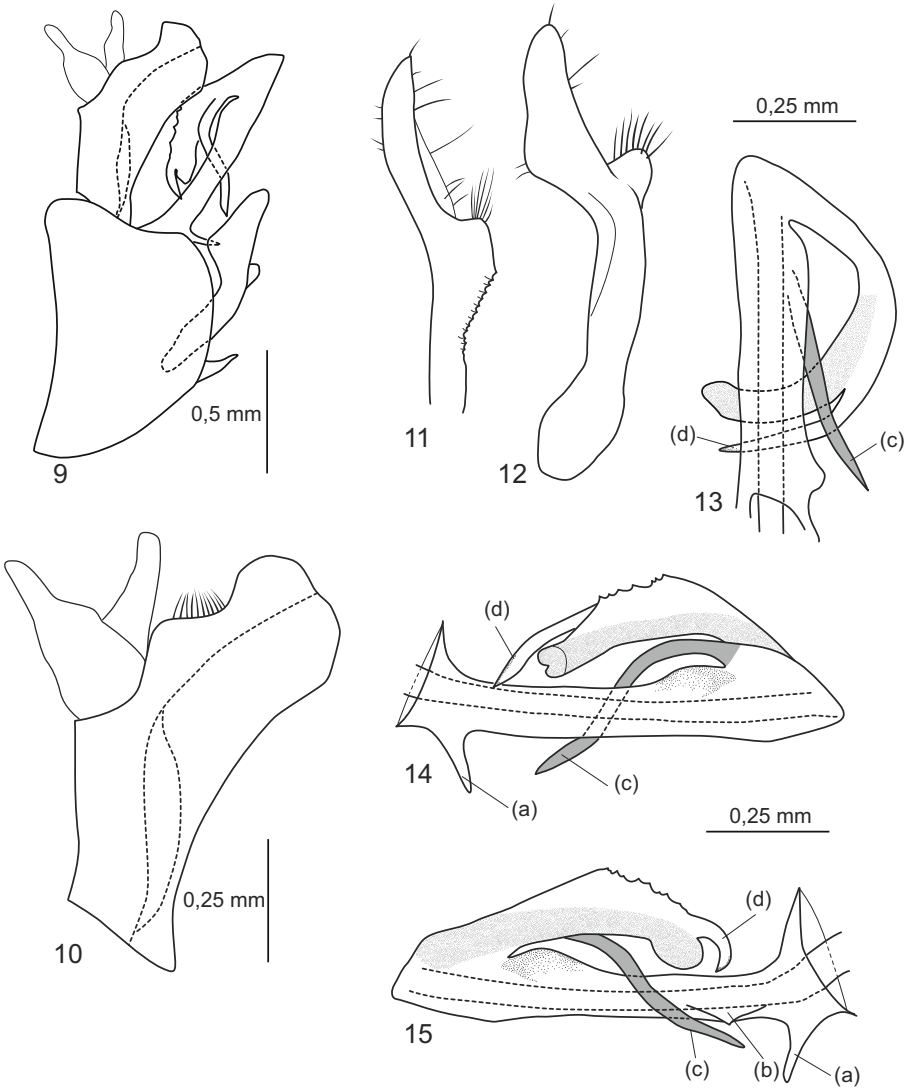
Male genitalia were re-drawn (Figs. 9, 10, 11, 12, 13, 14 and 15), to complete the original drawings by Synave (1953). In lateral view anal tube longer than wide, ventral process slightly longer than proximal part before epiproct (versus Synave). Pygofer symmetrical; in lateral view posterior margin dorsally concave before being ventrally convex. Medioventral process well developed. As in most cixiids the aedeagus s.s is situated in the continuity of a tube-like periandrium (Bourgoin et al. 1998); aedeagus s. l. asymmetrical: periandrium tube-like, elongated, with a ventral basal spine (a), a small and wide lateral tooth on the lateral left side (b) (versus Synave) and a long left apical acute process pointing antero-ventrally (c); aedeagus s.s. dorsally sclerotised, dorsal margin irregular, ventral and apical part membranous corresponding to the endosoma and a long antero-ventrally oriented aedeagal process (d). Gonostyli elongated, apically acute, with short submedian and ventral rounded process bearing setae.

Female habitus similar to male, larger. Female genitalia (Figs. 16, 17, 18 and 19) of orthopteroid type: gonapophyses VIII and IX and gonoplacs forming together a piercing–type sclerotised structure, typical for Cixiidae. Anal tube small and narrow, short in length; in lateral view ending abruptly after the



Figs. 4–8 Pictures, *4*) Aerial photograph of the Namoroka's Tsingy; *5*) The principal author conducting visual search for *T. namorokensis* on a "column" of roots in the cave; *6*) *Typhlobrixia namorokensis* nymphs on rootlets network; *7*) *Typhlobrixia namorokensis* adult; *8*) *Typhlobrixia namorokensis* nymph in its "nest", 4th or 5th instar. Photo taken by T. Bourgoin (*5* & *8*), M. Gansuana (*4*), D. Ouvrard (*7*) and A. Soulier-Perkins (*6*)

insertion point of epiproct and paraproct that surpass it posteriorly. Segmental and appendicular structures with gonocoxae VIII and tergum VIII well sclerotised (Fig. 16). Gonapophyses VIII well developed, elongate, lanceolate, joining ventrally to form a gutter-like structure. Gonapophyses IX, long and strongly sclerotised, fused together dorsally into a single structure, the suture still observable apically (Fig. 17). Gonoplacs well developed, large, dorso-ventrally flattened, covering the gonapophyses IX dorsally. Gonospiculum present but vaginal process absent as the gonospiculum bridge. Complex of the ectodermal genital ducts of monotrysian type as in all known Cixiidae. Sclerotised plates (SclV) in the first anterior half part of the wall and folds of the vagina, located close to the gonoporus. Vagina prolonged dorsally by a large bursa copulatrix, single chamber, completely ornate by small sclerotised micro-structures (Fig. 19). Common oviduct opening ventro-laterally in between the vagina and the spermatheca.

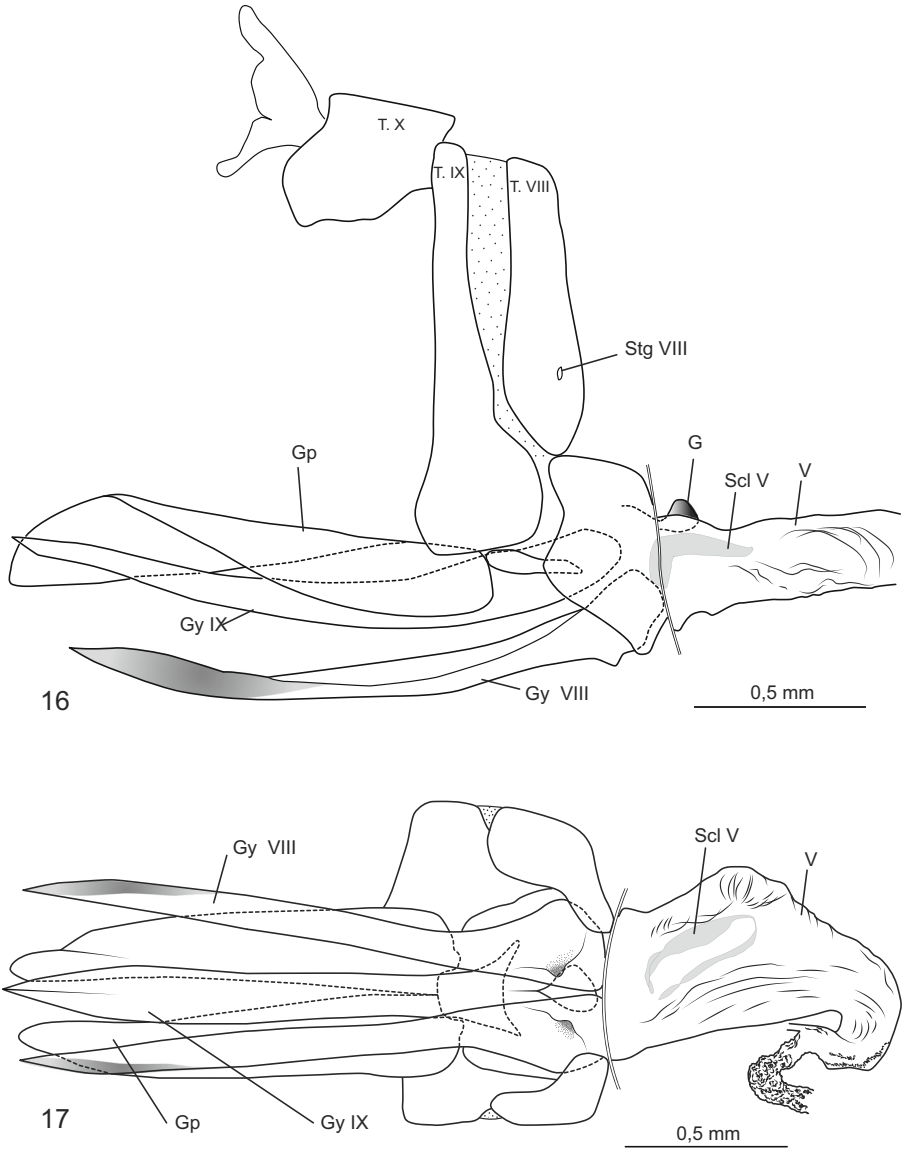


Figs. 9–15 *Typhlobrixia namorokensis* male *9*) genital capsule, lateral view; *10*) anal tube, lateral view; *11*) Gonostylus, ventral view; *12*) Gonostylus, lateral view; *13*) Aedeagus s. l., ventral view; *14*) Aedeagus s. l., left lateral view; *15*) Aedeagus s. l., right lateral view, **a**) ventral basal spine on periandrium; **b**) lateral tooth on lateral left side of periandrium; **c**) left apical process on periandrium; **d**) antero-ventral view of aedeagal process

One 5th instar nymph is illustrated in Synave (1953). For habitus of earlier instar nymphs see Fig. 6 (3rd instar) and Fig. 8 (4th instar).

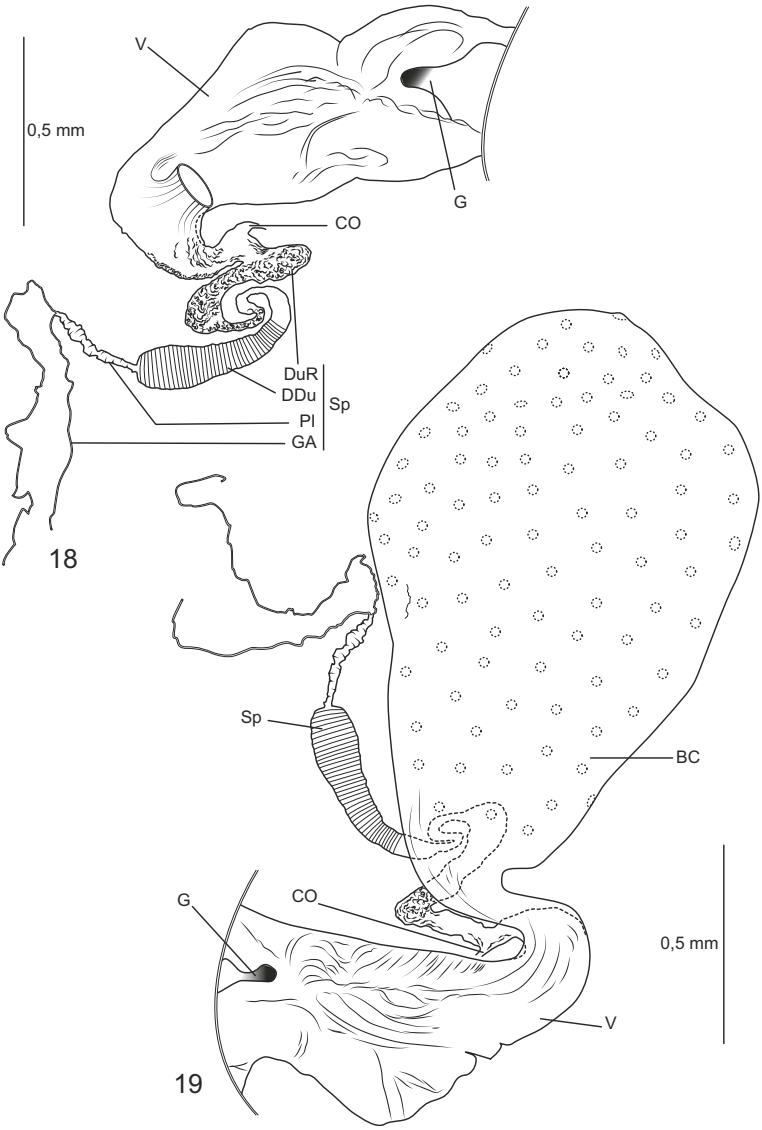
Molecular Data

The DNA was extracted from three nymphs and two adults. A portion of the 18S was successfully amplified and sequenced for the three nymphs but not for the adults. The



Figs. 16–17 *Typhlobrixia namorokensis* female *16*) External genitalia and vagina, lateral view; *17*) External genitalia and vagina, ventral view. *G* gonospiculum, *Gp* gonoplac, *Gy VIII & IX* gonapophyses VIII & IX, *Stg VIII* stigmata VIII, *Scl V* sclerite in vagina, *T* VIII-T, *X* tergites VIII to X, *V* vagina

total length of the sequences was 1296 and 1295 base pairs (sequence between helix 22 and helix 50) respectively for the specimens MNHN (EH) 19263 and 19265. For the third nymph MNHN (EH) 19272, only the region between helix 22 and helix 38 was sequenced, representing 786 base pairs, identical to the sequence of the specimen MNHN (EH) 19263. Both of these specimens were found in the cave network Ambovonomby along with the adults of *T. namorokensis*. Since their sequences were 0 % divergent it can be said that they are conspecific. Differences were observed for the



Figs. 18–19 *Typhlobrixia namorokensis* female *18*) Internal genitalia, ¾ above view right, the bursa copulatrix is not represented; *19*) Internal genitalia, ¾ above view left. *BC* bursa copulatrix, *CO* common oviduct, *DDu* diverticulum ductus, *DuR* ductus receptaculi, *G* gonospiculum, *GA* glandula apicalis, *PI* pars intermedialis, *Sp* spermatheca, *V* vagina

nymph collected in the network of Antsifotra (MNHN (EH) 19265): 2 transversions, 13 transitions and 1 deletion. The p-distance between this nymph and the other two is 1.23 %. The sequences are available on GenBank under the following accession numbers: KT602430, KT602431, and KT602432, corresponding respectively to the specimens MNHN (EH) 19272, 19263 and 19265. 689 base pairs of CO1 sequence were obtained from one individual, MNHN (EH) 19272 (from Ambovonomby network). This sequence is available on GenBank under the accession number KT602433.

Signal Analysis

Our first attempt at recording was not performed in the cave where the specimens were collected, but in a more accessible and open cave where we installed a small animal breeding container. The nymphs that we kept in this cave were fed for 10 days with potatoes and zucchini at 22–25°c at a humidity of 45–49 %; they reached the adult stage, but in this environment none of the adult specimens tested emitted any vibrational signal. After 2 days of trying, we moved further inside the cave where the specimens were collected, where the humidity was higher (73–83 %) and the temperature lower (19,9–20,4 °C). Only under those conditions, in an innermost section of the cave, did the specimens placed in the recording arena produce vibrational signals. Initially, we placed one male and a female in the container. When no signal was produced, we added a second female and three males. One specimen started to call (MNHN SO 2015-7) and after several minutes a second specimen called as well (MNHN SO 2015-15). We were successful in recording these communication signals.

The carrier frequency of the call is about 155 Hz. No significant modulation of amplitude or frequency was observed. The call was composed of a series of pulse trains, each train generally comprising three to four pulses (Figs. 20 and 21). Each pulse lasted 0.04 s±0.01 s. The interpulse interval (gap between each pulse within a train) varied between 0.07 and 0.09 s. In the case of a "typical" train containing 4 pulses, the train lasted around 0.4 to 0.5 s. In some cases the interpulse interval could be significantly shortened to 0.03 or even to nearly zero. In those cases, the train lasted less than 0.2 s. Within a calling period, the interval between each train was very variable and could fluctuate from 0.1 to 1.28 s. After a period during which a single specimen called, a second individual started to answer. As in the case of the first calling specimen, it was impossible to determine its sex. The calls alternated and never overlapped (Fig. 22). The call of the second specimen, further away from the recording site than the first calling specimen, could be distinguished by its smaller amplitude (Fig. 22). However, the structure of the call was the same.

Discussion

Implications on Malagasian Cave-Planthopper Diversity

Information on genetic distance, allowing one to tell if two specimens are within the range of intra or interspecific variation, are rare for the Hemiptera and available only for a few genes. Bressan *et al*. (2009) showed that the CO1 of the genus *Pentastiridius* (Hemiptera, Fulgoromorpha: Cixiidae) presents a genetic distance of 0.2 % for intraspecific variation and reaches 7.8 % for interspecific DNA divergence. In another study, Dijkstra et al. (2006) worked on the 12S and 16S genes of Delphacidae (Hemiptera: Fulgoromorpha). For 12S, populations in a single species differed by 0.3 % and the smallest interspecific divergences were 1.0 %. 16S showed a higher level of divergence, 1.1 % for intraspecific variation and 3.6 % for the smallest interspecific difference. Depending on the gene, the level of divergence required to separate two species is different. Consequently, no conclusion can be reached for the 18S we sequenced. For this reasons, we checked some available 18S sequences of

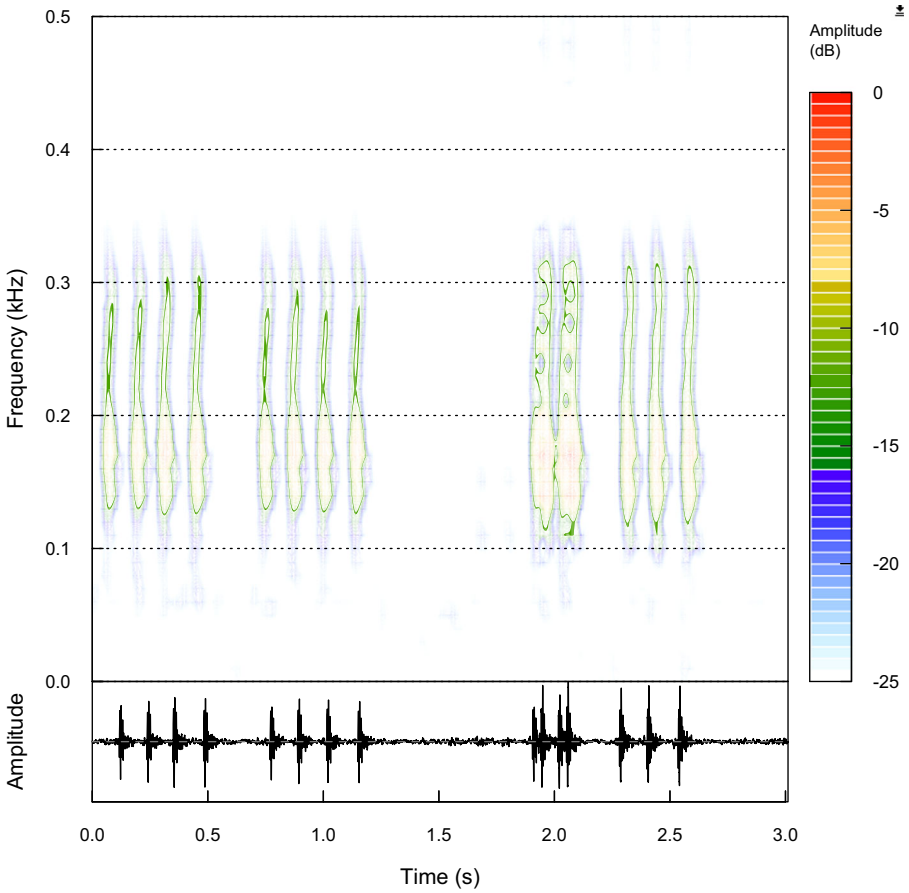


Fig. 20 *Typhlobrixia namorokensis*, four trains call. Spectrogram obtained with 512 sample FFT, Hamming window with 95 % overlap; frequency resolution of 9.77 Hz and 25 dB dynamic range; Oscillogram, the scale of Y axis is relative and without unit

Enchophora (Hemiptera, Fulgormorpha: Fugoridae) from a study by Urban and Cryan (2009). No differences are observed between the sequences of two varieties of *Enchophora sanguinea* Distant, 1887, but between the three species *E. sanguinea*, *Enchophora rosacea* Distant, 1887 and *Enchophora stillifera* (Stål 1862) divergence fluctuates from 0.34 to 0.85 %. The observed difference between the specimens collected in Antsifotra and Ambovonomby is 1.23 %. Therefore, we interpret the observed difference in 18S sequence to be sufficiently high to assume the non-conspecifity of the individuals from the two networks. While the specimens from Ambovonomby could be identified as *Typhlobrixia namorokensis*, the identity of the species from Antsifotra network remains unclear: adult males are mandatory for an unambiguous identification to species level. Those results are supported as well by morphological observations. The nymphs found in Antsifotra caves are much smaller in size (half the length) at a similar stage of development but already exhibit a few red ommatidia, whereas the nymphs at the same stage collected in Ambovonomby show no ommatidia. The nymphs found in Antsifotra seem very likely to belong to a different

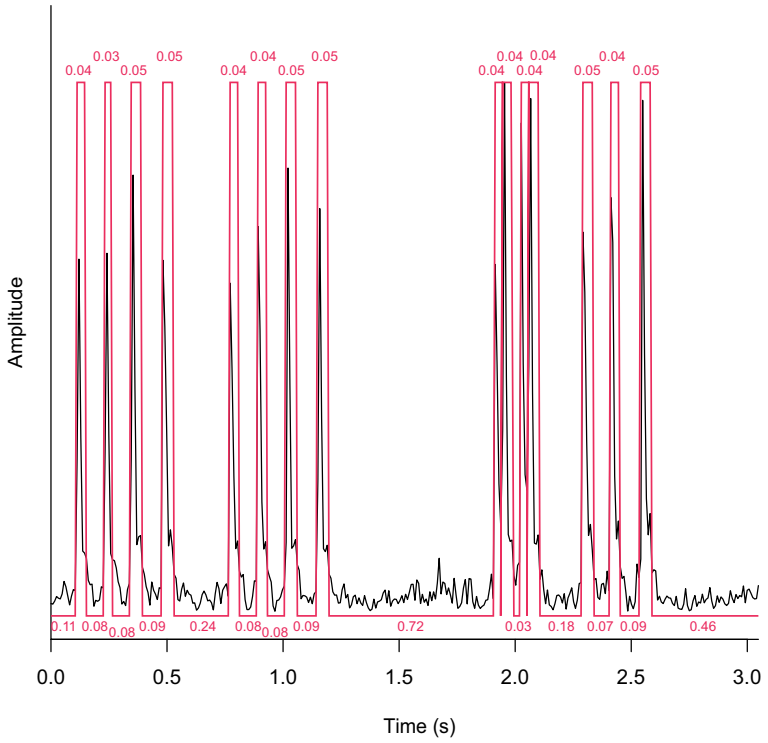


Fig. 21 *Typhlobrixia namorokensis*, measure of the temporal pattern of the signal in relationship to its amplitude

taxon but the identity of that species can only be assessed after adults have been collected. The COI sequence was amplified successfully only from a nymph from the Ambovonomby network where it was found together with adults of *T. namorokensis*. Considering the limited area explored in comparison to the size of the whole Namoroka Tsingy, the chances are high that yet undiscovered troglobitic planthopper species exist. As already postulated by Hoch et al. (2014: 932), "intensified efforts to explore the subterranean biome will doubtlessly reveal the existence of additional cave-dwelling planthopper taxa, and may eventually add Madagascar to the list of hot-spots of cave-planthopper diversity – next to the Canary Islands, Australia and Hawaii".

T. namorokensis Evolutionary History

Typhlobrixia is a monotypic genus, endemic to Namoroka, with no epigean species known. Seven other genera of Cixiidae are known from the island (Bourgoin 2015): *Achaemenes* Stål 1866, *Aselgeoides* Distant 1907, *Brixia* Stål 1856, *Eumyndus* Synave 1956, *Myndus* Stål 1862, *Nesomyndus* Jacobi 1917 and *Oliarus* Stål 1862, encompassing 63 species. The relationships between this genus and these other taxa have yet to be established since no phylogeny of the Malagasian cixiids is currently available.

At first, we could believe that this species underwent so many alterations during its evolutionary process of cave adaptation that when it was first

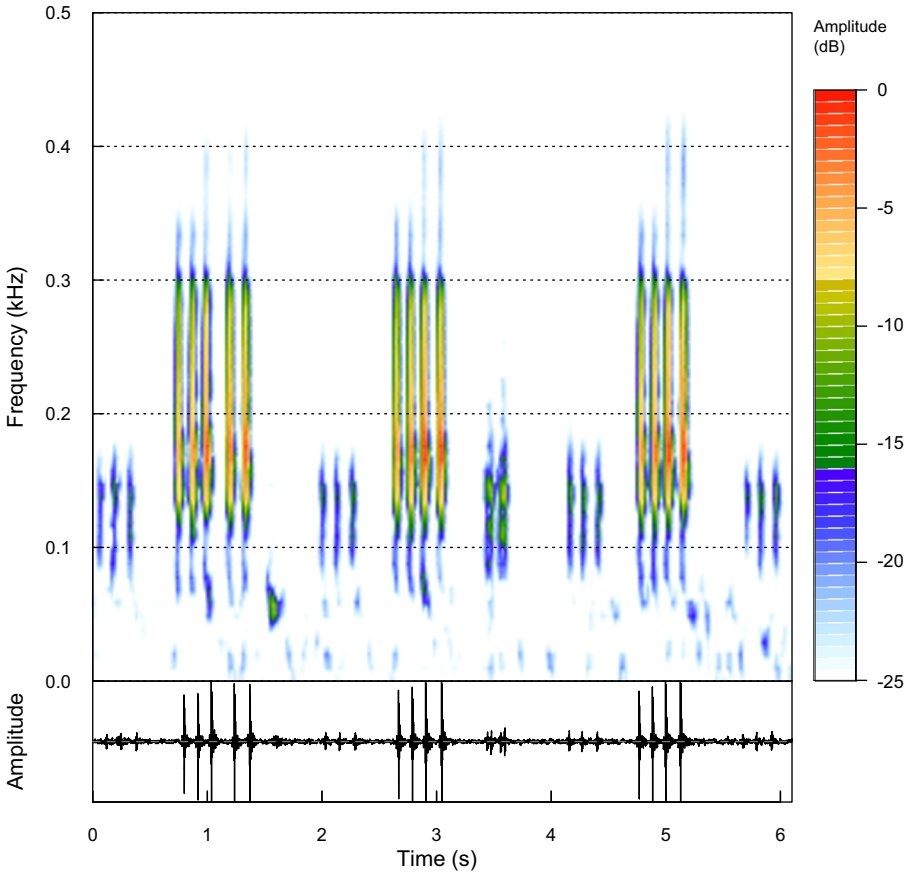


Fig. 22 *Typhlobrixia namorokensis*, 2 specimens alternately calling. obtained with 512 sample FFT, Hamming window with 95 % overlap; frequency resolution of 9.77 Hz and 25 dB dynamic range; Oscillogram, the scale of Y axis is relative and without unit

discovered and described, no character allowed the describer to place it within an existing genus. First, as we mention in the re-description, the taxon is not that highly transformed into a troglomorphic species. Second, when looking in the literature concerning genera containing species living in caves, we notice that even in some extreme troglomorphic cases such as highly modified *Solonaima baylissa* Hoch and Howarth 1989, we are still able to link this species to the existing genus *Solonaima* to which it belongs (Soulier-Perkins 2005). It seems likely that *T. namorokensis* was described in a different genus because enough characters clearly distinguished it from any other genus, and no epigean species belonging to this genus were yet known.

Therefore what could be hypothesised about the evolutionary history of this troglobitic species? Is it a relict, the result of a climatic crisis that drove part of an epigean population into the caves where it survived when the surface population disappeared, i.e., does the classic climatic relict hypothesis (CRH, Vandel 1964) apply here? Or is it rather a pathfinder, resulting from an adaptive shift, i.e., does the adaptive shift hypothesis (ASH, Howarth 1987) applies instead? Adaptive shifts may occur

when, e.g., previously unexploited food resources, such as roots penetrating the ceiling of the Tsingy caves, become available.

If the ancestor of *T. namorokensis* had to face an ecological and/or climatic crisis, we can hypothesise it took refuge in the caves where we now find the relict *T. namorokensis*. We can then expect that we will not find any sister epigean species, and *T. namorokensis* evolutionary history will best fit the CRH hypothesis.

Otherwise, an adaptive shift could be at the origin of the cavernicolous species. When the vegetation developed on top of the karst, the roots grew into the caves, offering a new biotope to sap-sucking insects. The ancestor of *T. namorokensis* invaded the caves and used these new unexploited food resources. Under this second hypothesis we should therefore expect to find a sister epigean species to *T. namorokensis*. However, the karst is currently surrounded by a dry and deciduous forest, dominated by sclerophyllous trees and shrubs, largely impacted by agricultural grazing and deforestation; the primary vegetation has been strongly reduced. The missing epigean *Typhlobrixia* relative might be therefore the result of those recent impacts on the biotope.

A similar situation is described for the only known cavernicolous Meenoplidae species, *Tsingya clarkei* Hoch et Wessel, 2014 (Hoch et al. 2014), and it is possible to elaborate evolutionary scenarios in the framework of CRH or ASH without credible evidence to support one more than another. Finding epigean and/or cavernicolous relatives could help on understanding better the factors that might have driven the evolution of those cave-adapted planthoppers on Madagascar. In the near future, a new field expedition will take place in the northwestern part of the Namoroka. From recent aerial photographs, some small islands of vegetation remain in this area, which could be refuges harboring an epigean representative of *Typhlobrixia*.

Adaptation to Caves and Behaviour of *T. namorokensis*

What is the degree of troglomorphy and ecological classification of *T. namorokensis*? The adults of *T. namorokensis* show some signs of troglomorphy such as the reduction of the compound eyes into a smaller number of red ommatidia. Body pigmentation of the individuals is lighter than in epigean species. As all nymphal instars (except first instar, which is very small) have been observed to occur inside the caves, it is assumed that *T. namorokensis* completes its entire life cycle in the cave. According to the definition given by Sket (2008) it is here classified as being a true troglobiont (i.e., an obligate cave dweller, completion of life cycle underground mandatory) and not an eutroglophile (i.e., an essentially epigean species able to maintain a permanent subterranean population) because it was only in deep subterranean conditions at low temperatures and high humidity that the adults were capable of initiating mating behaviour. As already mentioned by Hoch (2002) for other caves dwelling species, the quantity of wax produced by the adults and nymphs is important, covering part of their body and – as has been observed for *Oliarus polyphemus* from lava tubes in Hawaii – wax filaments are used to produce fluffy “cocoons” around the nymphs feeding on the roots. Also in *T. namorokensis* isolated and immobile nymphs were observed inside a kind of rounded “nest” made of this wax (Fig. 8). It remains to be determined whether the wax “nest” provides some protection from predation or if it is just a passive deposit.

Typhlobrixia namorokensis Communication

In order to survive in the caves, these organisms must not only find a sufficient quantity of food but they must be able to communicate, to meet and reproduce. In most of the caves we explored, numerous roots are hanging from the ceiling and a fine network of rootlets is running on most walls and anfractuosities, providing the support for insect communication (Fig. 6). During the recording, the insects were successively calling and seemed to "answer" to each other. However we cannot demonstrate that these calls were dedicated to locating potential sexual partners; it is equally conceivable that they were rivalry calls between individuals. The emissions of the vibrational signal were not accompanied by clear and visible movements of the abdomen, which made it impossible to say that only individuals of the same sex were singing or if there was alternation between specimens of both sexes. Nor can we conclude that the homogeneity observed in the pulse trains produced by all the specimens is due to the absence of sexual dimorphism in the call, because females and males were never observed duetting. Furthermore, during the recording, we did not observe any male and female approaching each other or mating as a result of the exchange of calls.

Analysis of the recording of this cavernicolous species in situ remains a preliminary result, but we can assert that the humidity and the temperature strongly influenced the behaviour of these insects. It was only when the humidity was high enough and the temperature low enough that they started to produce their vibrational signal. The call structure presents no significant modulation of amplitude or frequency: as described in the results, it is composed of a series of pulse trains, each train generally comprising three to four pulses. These sound like a succession of four simple clicks, a pause of 0.1 to 1.28 s, then again four clicks (Figs. 20 and 21). In Australia, the facultative cave species *Solonaima pholetor* Hoch and Howarth 1989 presents an even more simplified call structure with 1 click, 2 s of silence followed by another click (Hoch and Wessel 2006). For the obligate cave species *S. baylissa*, the call structure is more complex as described by Hoch and Wessel (2006): two initial chirps, a longer trill and a final pulse resembling the sound of a fog-horn. For the species complex found in Hawaii, *Oliarus polyphemus* Fennah 1973, even if some variation of the calls is noticeable between populations of the different caves, the courtship signal remains simple and roughly consists of a long series of similar pulses. Generally, the complexity of call structure for the cavernicolous planthoppers seems reduced when compared to the epigean species (Hoch and Wessel 2006). Considering that when competitive pressure of related species is lower the need to maintain a highly complex signal might not be as important, the difference in complexity between epigean and cavernicolous species could be the result of such ethological release (Booij 1982; Hoch 2000). *T. namorokensis* has been up to now the only cave-dwelling cixiid species found in the Namoroka caves; consequently the risk of interbreeding is low and there is perhaps no selection pressure to maintain a signal of high complexity. The situation is similar for the *S. pholetor* and *O. polyphemus* species complexes, which occur largely in allopatry. These species fit with the ethological release hypothesis. Concerning *S. baylissa* the situation is slightly different, since it is sympatric to another cixiid species, which may explain why it maintains such a highly complex calling signal.

Our field observations of the endemic *Typhlobrixia namorokensis*, although preliminary, have revealed interesting facts about the ecology and behaviour of this species.

Future field work is needed to learn more about the endemic and narrow-range cave faunas of Madagascar, which constitute a significant element of the island's microendemic biota. This information will be critical to put regions rich in caves and karst biospace on the list of high-priority conservation areas (Kremen et al. 2008) of Madagascar, and eventually assist in expansion of the current reserve network.

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