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CAVE CRICKET GENUS *TROGLOPHILUS* AS A MODEL FOR STUDYING FUNCTION AND EVOLUTION OF SENSORY SYSTEMS AND BEHAVIOUR

Nataša Stritih Peljhan

Nacionalni inštitut za biologijo, Oddelek za raziskave organizmov in ekosistemov,
Večna pot 111, SI-1000 Ljubljana; e-mail: natasa.stritih-peljhan@nib.si

Abstract - The European cave crickets *Troglophilus neglectus* and *T. cavicola* (Orthoptera: Ensifera; Rhaphidophoridae) represent an important insect model for studies of sensory systems and behaviour related to detection of exteroreceptive stimuli. The reason lies both in their specific life style and the absence of hearing organs and sound communication, which are intensively investigated in other Ensifera, in particular in crickets and bushcrickets. In the first and the most extensive part of the review, I describe studies of mechanosensory systems, communication and behaviour in *Troglophilus* related to detection of mechanical signals such as substrate-borne vibration, air currents and gravity. Following are studies of the specific scent organs and olfactory communication developed in these species. These systems show a mixture of ancestral and derived characters, not only in comparison to Ensifera and other orthopteroids, but also between the two investigated species, thus offering important functional and evolutionary insights.

Key words: sensory physiology, neuroanatomy, sensory evolution, biotremology, mechanoreception, vibration, olfaction, aggression

Izvleček - JAMSKE KOBILICE RODU *TROGLOPHILUS* KOT MODEL ZA ŠTUDIJE DELOVANJA IN EVOLUCIJE SENZORIČNIH SISTEMOV IN VEDENJA

Evropski vrsti jamskih kobilic *Troglophilus neglectus* in *T. cavicola* (Orthoptera: Ensifera; Rhaphidophoridae) predstavljata pomemben model za raziskave senzoričnih sistemov in vedenja žuželk, povezanega z zaznavo zunanjih dražljajev. Razlog za to je tako v njihovem specifičnem načinu življenja kot tudi v odsotnosti slušnih organov in zvočne komunikacije, ki so intenzivno raziskovani pri ostalih dolgotipalčnicah, predvsem murnih in kobilicah. V prvem in najobsežnejšem delu pregleda bom opisala raziskave senzoričnih sistemov, komunikacije in vedenja povezanega z zaznavo mehanskih dražljajev kot so vibracije podlage, zračni tok in gravitacija. Sledile bodo raziskave specifičnih organov za oddajanje vonja in s tem povezane komunikacije pri vrstah iz rodu *Troglophilus*. Ti sistemi jamskih kobilic kažejo mešanico predniških in izpeljanih lastnosti, a ne le v primerjavi z dolgotipalčnicami in ostalimi ravnokrilci, pač pa tudi med obravnavanima vrstama. S tem nam omogočajo pomemben vpogled v razumevanje tako njihovega delovanja kot evolucijskega razvoja.

KLJUČNE BESEDE: senzorična fiziologija, neuroanatomija, senzorična evolucija, biotremologija, mehanorecepcija, vibracije, voh, agresija

Introduction

Rhaphidophoridae, commonly called cave crickets or camel crickets, comprise a wingless and ecologically specialized group of Orthoptera (so. Ensifera) with most of the species adapted to a certain degree of cave life (DiRusso and Sbordoni, 1998). They have been often considered a relic ensiferan lineage for their morphology (e.g. Ander, 1939; Desutter-Grandcolas, 2003) and the wide disjunct distribution across temperate areas of both hemispheres (Hubbel and Norton, 1978). In the still unresolved phylogeny of the Ensifera, however, different approaches placed cave crickets at various branch points of the group (e.g. Legendre et al., 2010; Song et al., 2015). Yet, regardless of phylogenetic position, their sensory systems and behaviour are very interesting to study comparatively with respect to other Ensifera. The reason is not only in their specific life habits but also in the absence of auditory communication and hearing, which is present and extensively studied in crickets and bushcrickets.

The genus *Troglophilus*, in the monotypic subfamily Troglophilinae, is distributed in the Eastern Mediterranean with at least 14 species showing one centre of species richness in the Balkans and the second one in the southern part of Asia Minor, including a part of the Aegean islands (Karaman et al., 2011). *T. neglectus* and *T. cavicola* are the most widely distributed European species that reach over Slovenia to Austria and Italy at the most north-western border of their area (Karaman et al., 2011). They represent the most abundant arthropods in the Slovenian karstic undergrounds, where both species often appear syntopically (Novak and Kuštor, 1983). As suggested already by the genus name, *Troglophilus* is not strictly bound to the underground habitats. The animals overwinter in deep cave parts, while in summer they are nightly active in the forests and use superficial parts of the caves and other endogenous forest places only as daily shelters (Novak and Kuštor, 1983; Karaman et al., 2011; Fig. 1). The life cycle investigated for *T. neglectus* and *T. cavicola* shows strict seasonality, a new generation each year, and is completed in two to two and a half years (Pehani et al., 1997). There is a temporal shift between the species, with *T. cavicola* mating in the early spring and *T. neglectus* in the late summer (Pehani et al., 1997; Stritih and Čokl, 2012). Consequently, the adults of one or another species may be encountered in caves literally throughout the year, which makes them continuously accessible for investigations. In the present review I describe studies of their

Fig. 1: *T. neglectus* male during a summer day, residing on a wall of an artificial tunnel (in Brje pri Komnu, SW Slovenia) close to its entrance. Scale bar = 10 mm.

sensory systems and the related behaviours, which were initiated already at the beginning of the previous century (Seliškar, 1923).

In the first part of the review, I describe studies of mechanosensory systems, communication and behaviours in *Troglophilus* related to detection of various mechanical signals, such as substrate-borne vibrations, air currents and gravity. Following are studies of the specific scent organs and olfactory communication developed in these species. The majority of research in both fields was conducted at the National Institute of Biology in Ljubljana (former Institute of Biology). Also the scientific career of Acad. Prof. Dr. Matija Gogala, to whom this special issue of AES is dedicated, started at this institute, with the research of vision particularly in *Troglophilus* cave crickets. In his doctoral dissertation, prof. Gogala demonstrated that compound eyes of these insects are normally developed and functional (Gogala, 1964; 1966). And although visual signals cannot be of much use for communication or orientation of a nightly active species such as is *Troglophilus*, they may be considered important for maintaining their activity rhythm related to daily migrations between the hypogean and the epigean habitats.

Mechanosensory systems

Detection, production and responses to substrate vibration

Due to the lack of stridulatory structures and supported by the existing data on mating behaviour, Rhaphidophoridae appear unable to produce audible sound (see Stritih and Čokl, 2012; Stritih and Strauß, 2015). They do, however, produce substrate-borne vibratory signals during sexual communication, which was demonstrated for the first time in *Troglophilus* cave crickets (Stritih and Čokl, 2012). In this respect, the mechanosensory complex scolopidial organ for sound and/or vibration detection in the legs of ensiferan insects (Lakes-Harland and Strauß, 2014) was investigated in *Troglophilus* for the presence and inter-specific homology of individual groups of sensilla (i.e. sensory organs; Jeram et al., 1995; Strauß et al., 2014; Strauß and Stritih, 2016), sensitivity to sound and vibration (Jeram et al., 1995; Čokl et al., 1995), morphology and central projections of sensory neurons (Stritih and Čokl, 2014; Stritih and Stumpner, 2009; Buh, 2011; Stritih Peljhan et al., submitted), anatomy and function of postsynaptic neurons in the ventral nerve cord and their homology to auditory network elements (Stritih, 2009; Stritih and Stumpner, 2009), as well as the mechanical response of the legs and the body that filter the received signals prior sensory transduction (Stritih Peljhan and Strauß, 2018; Stritih Peljhan et al., submitted).

The subgenual organ complex

While the majority of insects possess only the subgenual organ in the proximal tibiae as the major vibrosensitive organ (albeit with highly varying complexity), orthopteroids developed an especially complex tibial mechanoreceptor system with up to 4 different sound and/or vibration sensitive scolopidial organs in close proximity (Lakes-Harlan and Strauß, 2014). In this “tibial organ” in the legs of cave crickets (Jeram et al., 1995), more recently called also the “subgenual organ complex” (SGOC; Strauß et al., 2014), the existence of two major groups of scolopidial sensilla was demonstrated by the initial histological study: the subgenual organ (SGO) with ca. 30 scolopidial sensilla and the intermediate organ (IO) with 13−15 sensilla, showing a similar structure to that in bushcrickets (Jeram et al., 1995). No accessory auditory structures like external tympana or internal tracheal modifications for sound reception and transmission were found, and no sensilla at the distal location in the organ to suggest homology to auditory sensilla of Ensifera. Summed responses to auditory stimuli recorded from the leg nerve had very high thresholds compared to species with tympana and were restricted to low-frequencies (Jeram et al., 1995). In an accompanying study, the individual SGO and IO sensilla showed a high sensitivity to substrate vibration (and weak or no responses to airborne sound; Čokl et al., 1995; see also below). However, given that a relic (or reduced) auditory organ (called the “*crista acustica* homologue”, CAH), the third major structure in the complex, was subsequently found in all other major groups of Ensifera lacking tympana (Strauß and Lakes-Harlan, 2008a; 2008b; 2010), its putative presence in *Troglophilus* was reinvestigated, giving further anatomical details on neuronal innervation of sensilla (Strauß et al., 2014). The study confirmed the presence of only two major organs in the anterior SGOC, consistent with an ancestral organisation such as seen in outgroup Orthopteroidea (Strauß et al., 2014; Fig. 2A). Furthermore, the axonal tracing method revealed an additional small scolopidial organ posteriorly in the tibia. This is the accessory organ (AO), comprising a distinct cluster of 6−8 sensilla just next to the posterior hypodermis and the SGO (Strauß and Stritih, 2016; Fig. 2B). This organ is found to occur irregularly across orthopteroids (Strauß 2017).

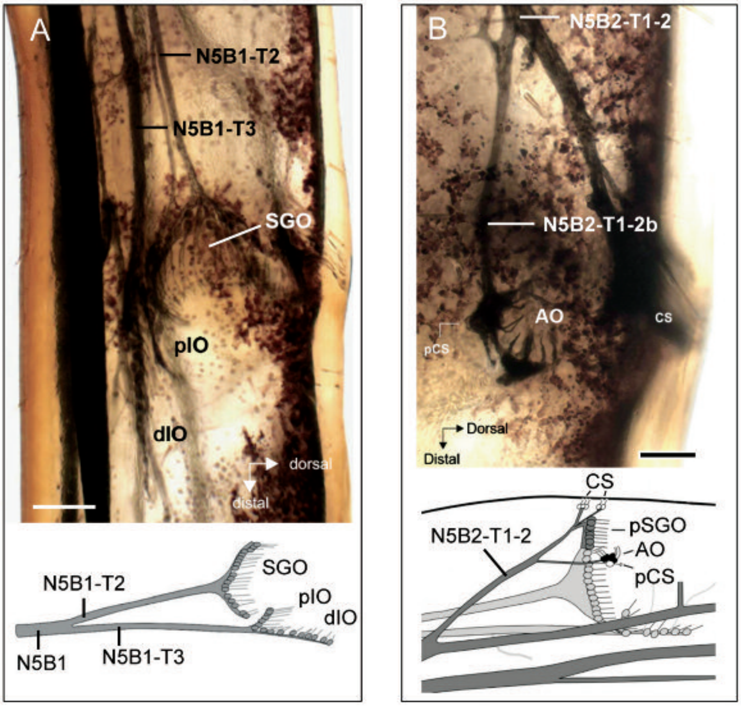

Fig. 2: Neuroanatomy of the SGOC in *T. neglectus* in wholemount preparations (above) and drawing reconstructions of innervation (below). **A)** Sensory elements innervated by nerve 5B1 (branches N5B1-T2 and -T3), anteriorly in the tibia. Above: midleg preparation, scale bar 100 µm. Below: the consensus branching pattern of N5B1, consistent with the presence of only two organs in the anterior SGOC; the SGO and the IO. **B)** Sensory elements innervated by nerve 5B2 (branch N5B2-T1-2), posteriorly in the tibia. Above: foreleg preparation, scale bar 50 µm. In the scheme below, elements innervated by N5B1 are shown at the back, and the upper line represents leg cuticle. The innervation schemes are not to scale, and are shown in 90° counter-clockwise orientation. Abbreviations: AO – accessory organ, CS – campaniform sensilla, pCS –posterior campaniform sensillum, pIO – proximal intermediate organ, dIO – distal intermediate organ, SGO – subgenual organ. Adapted from: A) Strauß et al. (2014) and B) Strauß and Stritih (2016), the latter with permission from John Wiley & Sons.

The physiological responses of individual receptor neurons from the SGO and the IO in *T. neglectus* were extensively investigated by extracellular recordings (Čokl et al., 1995). Described were eleven functional receptor types, four with best sensitivity to vibration between 700 and 2000 Hz and the rest with a tuning to lower frequencies (200−700 Hz), supposedly originating in the IO and the SGO, respectively (Čokl et al., 1995). This presumption was based on the comparison to bushcrickets that show

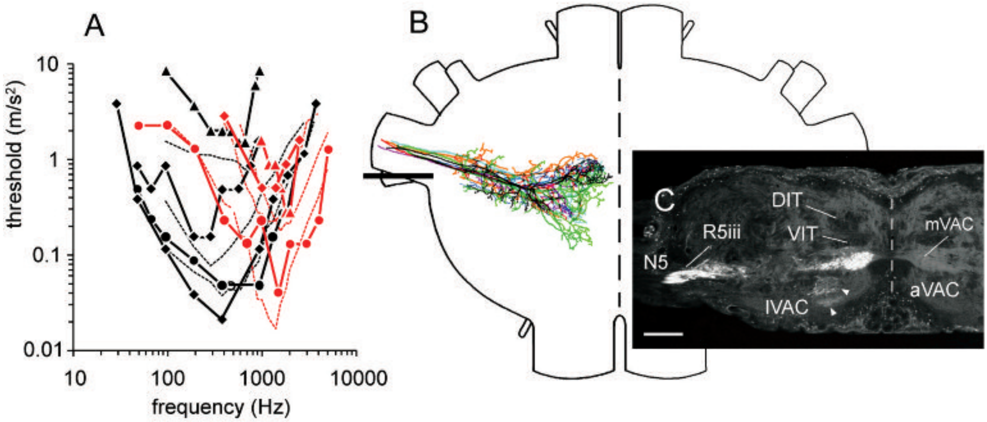

Fig. 3: Physiology, morphology and anatomy of central axonal projections of vibratory receptor neurons from the anterior SGOC of *T. neglectus*. **A)** Threshold tuning curves for seven types of intracellularly recorded receptors from forelegs (full lines with symbols), with corresponding tuning curves of the extracellularly recorded response types (dashed lines). Black: SGO receptors, red: IO receptors. **B)** Terminal arborisations of the intracellularly stained receptor neurons in the prothoracic ganglion (wholemount dorso-ventral view). Individual neurons from different preparations are shown in different colour and superimposed. The assembly shows a typical bifurcation pattern, revealed also by the anterograde tracing of N5B1 axons. **C)** Anatomical location of axonal projections from N5B1, filled anterogradely with Lucifer Yellow, in the transverse section of the ganglion at the level indicated in B). Abbreviations: DIT, dorsal intermediate tract; VIT, ventral intermediate tract; aVAC, anterio-ventral association centre; mVAC, medio-ventral association centre; N5, nerve 5 (= main leg nerve); R5iii, third root of the leg nerve. New data analysis is shown in B), while A) and C) are adapted from Stritih and Čokl (2014), with permission from Springer, and Strauß et al. (2014, respectively).

similar vibratory receptor classes, of which the IO receptors show the response also to low-frequency sound due to their functional coupling to the auditory apparatus (Kalmring et al., 1994). The constraint of these studies, however, was that neither payed attention to responses tuned to frequencies below 200 Hz, a priori considering them as a response of proprioreceptors and thus less important for complex sensory processing. In following investigations, vibratory receptor neurons were studied in *Troglophilus* using combined intracellular recording and staining, which allows for a morphological characterisation of the terminal branching pattern and anatomical position or the recorded axons in the neuropile (Stritih and Stumpner, 2009; Buh, 2011; Stritih and Čokl, 2014; Stritih Peljhan et al., submitted). These studies showed that the high-frequency tuned receptors conforming to physiological classes of Čokl et al. (1995; Fig. 3A), which were identified most frequently, project ventrally into the medio-ventral association centre (mVAC) of the segmental ganglion − the region specialised for processing of auditory and vibratory inputs (e.g. Strauß et al., 2014; Fig. 3B, C). An additional low-frequency receptor neuron type with a tuning to 50−300 Hz and projections into the dorsal part of the mVAC was described for the first time as a part of the SGO complex, and originates either in the AO or the posterior SGO, based on anatomical and physiological characteristics (Stritih Peljhan et al., submitted). This provides the first intracellular data on the sensory input into the region of the ventral nerve cord devoted to processing of low-frequency vibratory stimuli, such as are used in communication of *Troglophilus* and many other orthopteroids (Stritih and Čokl, 2014; see also below).

Mechanical leg response

Detection of substrate vibration is related to exceptionally strong physical constraints of the transmission medium that filters and distorts the propagating signals. Using laser vibrometry, we have investigated the influence of mechanical properties of the legs in *Troglophilus*, the finite element in signal transmission, on the sensory adaptations of the individual organs in the SGOC (Stritih Peljhan and Strauß, 2018; Stritih Peljhan et al., submitted). The tibia was found to be the most appropriate place for vibrosensory organs, since it vibrated stronger than any other leg segments in the response to substrate vibration due to mechanical resonance (Fig. 4A). Position of the response peak depended largely on various parameters of the stance, such as the level of leg flexion (Fig. 4B), the presence of body-substrate contact and apparently also the muscle tension, which gives the animals a potential to strongly influence vibration detection by postural adjustments. The response intensity increased with the increasing stimulus frequency (Fig. 4C), and the shape of the response function peaking at high frequencies matched the threshold curves of two IO receptor types from *Troglophilus* (Čokl et al., 1995) closely (Fig. 4D). These data suggest an adaptive value of the IO in detecting high frequency vibration transmitted over the leg surface (Stritih Peljhan and Strauß, 2018). Such a function would further distinguish the IO from the SGO, which is excited by haemolymph movements within the tibia caused by substrate vibration (Kilpinen and Storm, 1997). In the following set of measurements we compared the mechanical response between the lateral sides of the proximal tibia, showing that in the narrow low-frequency range between 100 and 200 Hz the surface of the posterior tibia oscillates slightly, but significantly, more intensely than the anterior tibia. This difference indicates that the specific position of the AO, linked to the posterior cuticle, may be adaptive (Stritih Peljhan et al., submitted).

Anatomy, function and homology of vibratory interneurons

When a sensory organ changes functionally during evolution, what happens to the central neuronal network receiving inputs from this organ? This question directed our research of vibration-sensitive interneurons in the prothoracic ventral nerve chord ganglion of *T. neglectus* (Stritih and Stumpner, 2009; Stritih, 2009). Having in mind the conservative nature of the central neural system, we expected to find homologues to the auditory neurons of Ensifera, which are in the hearing species connected to the functionally elaborated sound- and vibration-sensitive organ in the forelegs. Among the 26

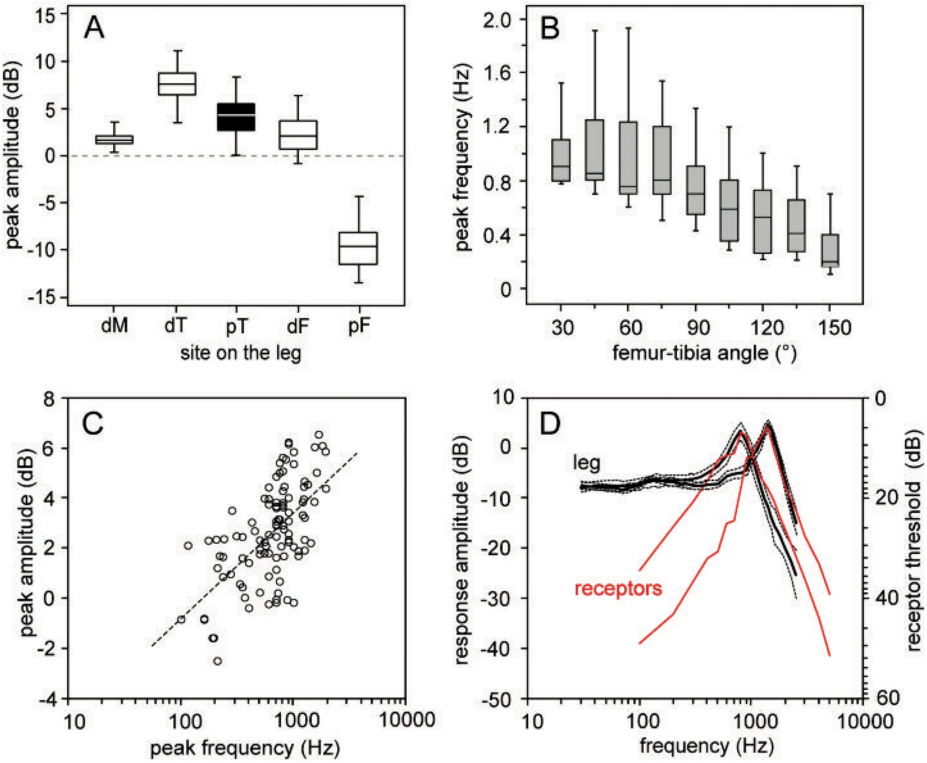

Fig. 4: The mechanical leg response to vibratory stimuli in *T. neglectus*. **A)** Response gain at different measurement sites of the leg (dM – distal metatarsus, dT – distal tibia, pT – proximal tibia (SGOC location; in black), dF – distal femur, pF – proximal femur), with the femur and tibia standing at right angles. Dashed line at 0 dB indicates the intensity of stimuli applied at the tarsus. **B)** Peak response frequency at different femur-tibia angles and **C)** peak response amplitude as a function of frequency from the same data set. **D)** Leg frequency-response functions (black lines, mean with S.E. range; left scale) for the legs/positions showing the peak at 800 Hz and 1400 Hz, superimposed to the mean (inversed) threshold curves of two IO receptor types tuned to these frequencies (red lines; right scale, shown in relative values – the intensity of threshold curves is set to the best match with the leg response). Adapted from Stritih Peljhan and Strauß (2018), with permission from Springer.

neuron types identified by their morphology and/or physiology, we recognised homologues to some of the specialised first-order auditory interneurons of crickets and bushcrickets (Stritih and Stumpner, 2009; Fig. 5). While retaining the same morphology of primary branches and soma location, similar intrinsic properties, and apparently also their basic implementation in the network, the auditory neurons showed drastic changes in dendritic morphology compared to their vibratory counterparts (Fig. 5A). Clearly, these changes reflect the modified receptor input from the vibratory to the auditory

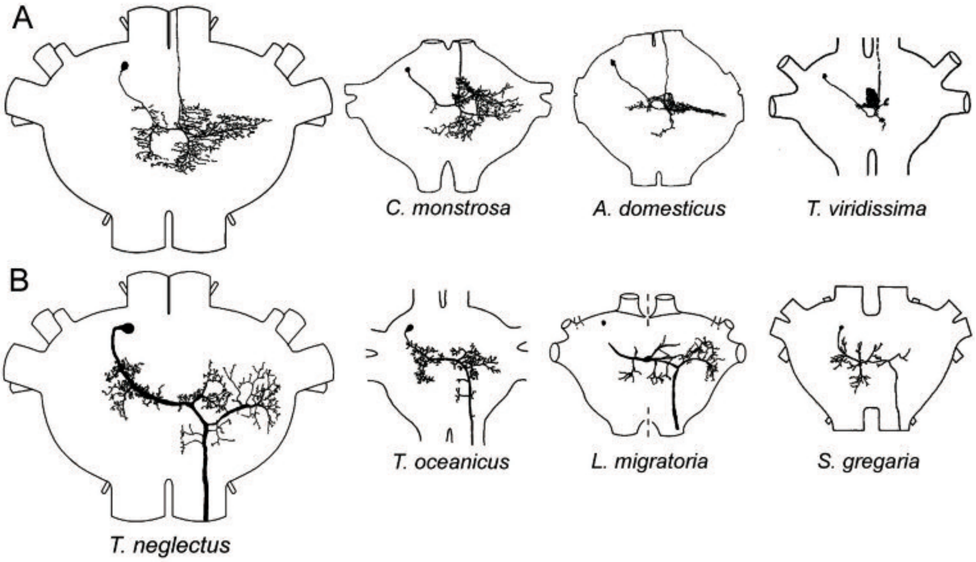

Fig. 5: Morphology of vibration-sensitive interneurons in the prothoracic ganglion of *T. neglectus*, with putative homologues from different Orthoptera (the hump backed cricket *Cyphoderris monstrosa*, crickets *Acheta domesticus* and *Teleogryllus oceanicus*, and the bushcricket *Tettigonia viridissima* of Ensifera, and the grashoppers *Locusta migratoria* and *Schistocerca gregaria* of Caelifera). The homologues in **A)** indicate the presumed evolutionary changes in morphology (from left to right) following auditory specialization from a vibrosensitive precursor neuron. The homologues in **B**) represent morphologically and apparently also functionally preserved neurons in the different lineages. Adapted from Stritih and Stumpner (2009), with permission from Elsevier. The neuron of *S. gregaria* is included additionally from Stritih (2006). The wholemount morphology of *T. neglectus* neuron in A) was reconstructed *de novo* from the original material (photos of histological sections).

portion of the sensory organ and the modified requirements for directional processing between the systems (Stritih and Stumpner, 2009). Again, in *Troglophilus* these neurons conform more to a primeval rather than reduced sensory organisation. Several further cave cricket neurons were homologised to neurons from various orthopteroids, where they often receive multimodal inputs and show comparatively little or no change in morphology and function (Fig. 5B). They were suggested as elements of the evolutionary conserved multimodal escape or warning system (Stritih and Stumpner, 2009).

From the functional viewpoint, an unexpected bias to processing of low frequency vibration inputs was found in *Troglophilus*, with the majority of interneurons responding most sensitively to vibration below 400 Hz (Stritih, 2009; Fig. 6A). While responses and neuropile location in a portion of these neurons suggested inputs from proprioceptive organs, a group of highly-sensitive neurons had dendritic (i.e. postsynaptic) segments in the mVAC neuropile specialised for processing of auditory and

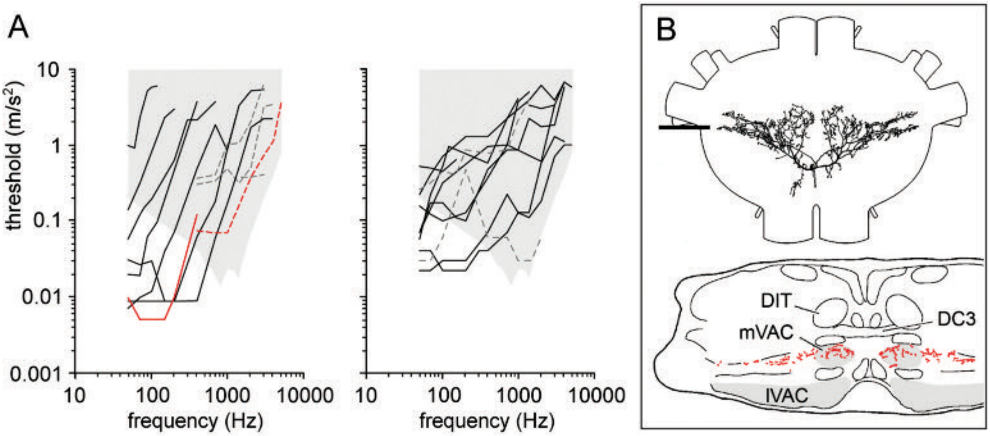

Fig. 6: Processing of low-frequency stimuli in the vibratory system of *T. neglectus*. **A)** Threshold tuning of 15 interneuron types with the best sensitivity to vibration below 400 Hz (full lines–excitation, dashed lines–inhibition). Neurons are shown in two classes according to the shapes of tuning curves. The gray area shows the response range of SGOC receptors (after Čokl et al., 1995). Note the the miss-match to interneurons, apparently with vibratory inputs from other receptor elements. **B)** Morphology of a highly-sensitive first-order interneuron (above; wholemount dorso-ventral view) and its location in the neuropile in the transverse section of the ganglion (below; neuronal branches are shown in red) at the level indicated above. In A), thresholds of this neuron type are marked red. Adapted from: A) Stritih (2009), with permission from John Wiley & Sons, and B) Stritih and Stumpner (2009), with permission from Elsevier.

vibratory stimuli. Dorsal location of some neurons in this neuropile (Fig. 6B) is consistent with inputs from a part of the femoral chordotonal organ, the posterior-most portion of the SGO and/or the AO sensilla (Stritih, 2009; see also Nishino, 2003; Nishino and Field, 2003). Such information processing was later shown to conform to processing of low-frequency vibratory tremulation signals in *Troglophilus* (Stritih and Čokl, 2012), signals that are often a part of multimodal courtship displays in other orthopteroids as well (Stritih and Čokl, 2014).

Mating behaviour and vibratory signalling

The high ecological similarity of *T. neglectus* and *T. cavicola*, together with their complete temporal isolation (Novak and Kuštor, 1983; Pehani et al., 1997), generally suggests a weak selection pressure for their divergent behavioural evolution. It was thus surprising to find differences in their mating behaviour, not only in the patterns or timing of principally similar behaviours, but extending to the level of employed stimulus modalities (Stritih and Čokl, 2012). During the close range courtship, *T. neglectus* males emit low-frequency vibratory signals by abdominal tremulation (i.e. oscillation without contacting the substrate; Fig. 7A, B), while such signalling is absent prior to pair formation in *T. cavicola* (Fig. 7C). In this species, the phase of mutual antennal fencing of the partners is much longer than in *T. neglectus*, suggesting a primary importance of tactile stimuli in courtship. And while the complete mating process only lasts a few minutes in *T. neglectus*, about 10-20 minutes is typical for *T. cavicola* (Fig. 7C). We suggested these differences to reflect the divergent mating habitats in and outside caves for *T. cavicola* and *T. neglectus*, respectively, which comply with timing of their respective sexual maturation immediately after and long after completed diapause (Stritih and Čokl, 2012). In spring one can frequently encounter female *T.*

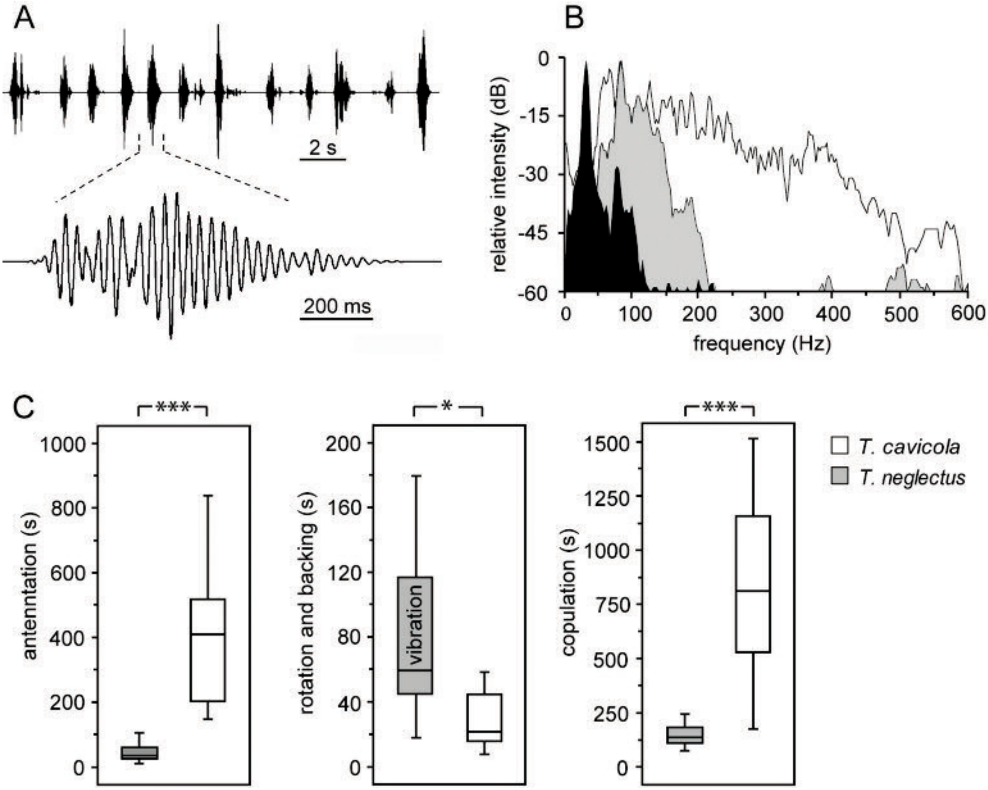

Fig. 7: Mating behaviour and vibratory signalling. **A)** Sample oscillograms of male vibratory courtship signals emitted by abdominal tremulation in *T. neglectus*. **B)** Sample spectrograms of these signals recorded from different substrates (black – elm bark, grey –spruce bark, white – moss). **C)** Duration of main phases of the mating process (appearing in succession from left to right) compared between *T. neglectus* and *T. cavicola*. Asterisks indicate different degrees of statistical significance (for more details see Stritih and Čokl, 2012). Vibrational signalling in courtship is expressed in *T. neglectus* only (see the middle diagram). After copulation, males of both species express vigorous whole-body tremulation with an unknown function (not shown). Adapted from Stritih and Čokl (2012).

cavicola with spermatophores still in deep cave parts, and its prolonged copulation possibly reflects the relaxed predation pressure in a cave environment. Since rock was proved extremely inefficient for vibration transmission (Stritih and Čokl, 2012; see also Stritih and Strauß, 2015; Strauß and Stritih 2017), we suggested vibratory signalling to be reduced in *T. cavicola* following its adaptations to mating underground. The sexual behaviour of *T. neglectus*, on the other hand, was suggested to represent a primitive condition of the sylvicolous cave cricket ancestors (Stritih and Čokl, 2012; see also Hubbel and Norton, 1978). In line with this hypothesis, recent data indicate that the genus *Troglophilus* diverged from the common ancestor very early in the evolution of Rhaphidophoridae (Song et al., 2015; Zhou et al., 2017) and may have thus retained many primitive characters. Its vibratory signalling may also represent the primitive mode of mechanosensory communication for the Ensifera, in which the different ways of vibratory signalling seen in the extant taxa could have been easily derived from abdominal tremulation (Stritih and Čokl, 2012; Stritih and Strauß, 2015).

A further divergence in the sexual behaviour of the two species relates to olfactory signalling and agonistic behaviour. An extensive reliance on odour has been demonstrated for *T. neglectus* males in agonistic contests (Stritih, 2014; Stritih and Žunič-Kosi, 2017; see also below). In *T. cavicola*, not only are their scent glands much less developed and were never observed exposed to the body surface as in *T. neglectus*, the species shows also no sign of inter-male aggression in the mating period (Stritih and Čokl, 2012). For cavernicolous animals aggression may be completely reduced to optimise energy expenditure (Ellipot et al., 2013), which is in line with a higher level of cavernicolous adaptations proposed for *T. cavicola*.

Behavioural responses to vibration

Despite the courtship vibratory signalling of *T. neglectus* males not being a subject of systematic experimental manipulation, its function in increasing female sexual receptivity and inducing her mounting for copulation may generally be assumed. This is also supported by far the longest vibratory courtship phase recorded in the pair that mated on the stony substrate in our study, on which the intensity of vibratory signals was below the vibrosensory detection threshold of the species (Stritih and Čokl, 2012; Stritih and Strauß, 2015). A clear behavioural response to vibration can be induced, on the other hand, by low frequency vibratory stimuli delivered to cave crickets at very high intensities. A stationary startle response, expressed as a jerky contraction of the legs and the body, occurs with a sharp tuning to 30−50 Hz vibrations that follow the line of equal stimulus displacement with the increasing stimulus frequency (Stritih and Čokl, 2014). The reaction, supposed to function as preparatory behaviour for the escape jump, is induced by almost the same frequency-intensity range of vibratory stimuli in locusts (Friedel, 1999). This behavioural agreement provides a further argument for the evolutionary conserved escape neuron networks of orthopteroids.

Detection and responses to air currents and gravity

As in other insects, the cave cricket's escape behaviour is multimodally triggered, but specifically for their life style it is strongly influenced by inclination of the ground. Anyone working with cave crickets would immediately notice how easy it is to collect them from cave walls, while on horizontal surfaces they typically show a high level of arousal. The inclination-sensitive gating of their locomotor activity and ventilatory movements was studied extensively by Kastberger (1982; 1984; 1985). He showed that the jump rate in *T. cavicola* stimulated by air puffs or touch decreases with the increasing steepness of the floor up to 60° and is almost completely inhibited on steeper surfaces (Fig. 8A). Along with jumping, cave crickets show thigmotactic behaviour (i.e. seeking shelters) that is positive on horizontal and negative on vertical

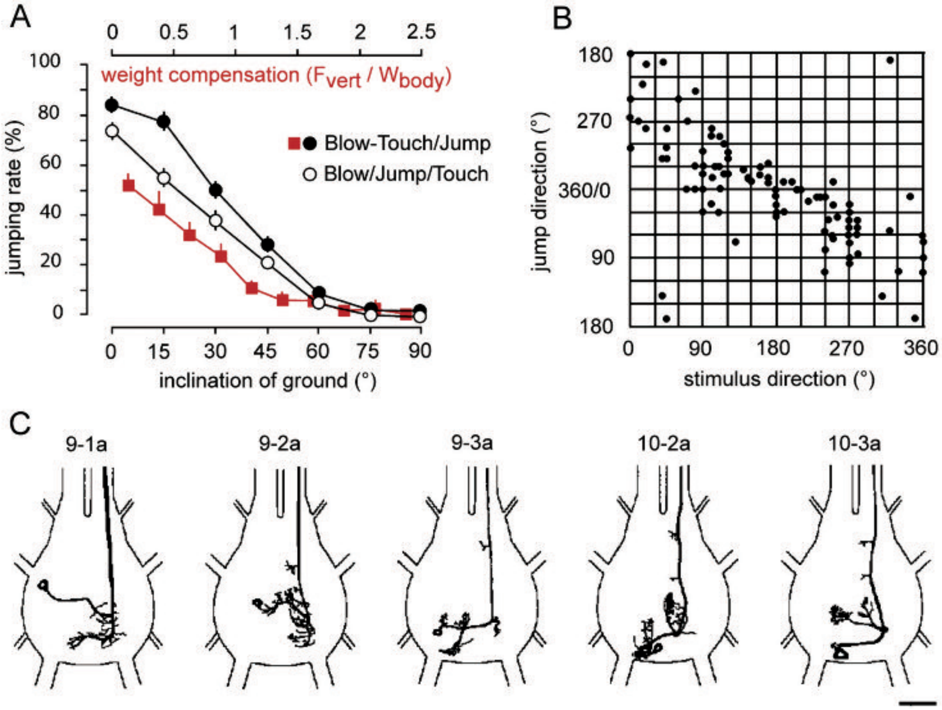

Fig. 8: Evasive behaviour, its proprioceptive gating, and giant interneurons of the cercal system. **A)** Dependence of the rate of evasive jumping in *T. cavicola* on the ground inclination (with two stimulus modes; black and white symbols, lower scale) and on compensation for body weight (red symbols, upper scale; F_{vert} –vertical force that compensates for body weight–W_{body}). Redrawn and combined from Kastberger (1982) and Kastberger (1984), with permission from John Wiley & Sons. **B)** Direction of the evasive jump in *T. neglectus* to wind puffs (0/360° - front of the animal, 180° - rear of the animal). Redrawn from Schrader (2000), with permission from Springer. **C)** Morphology of five types of GIs in the terminal abdominal ganglion of *T. neglectus* (wholemount dorso-ventral view) receiving mechanosensory inputs from the cerci. Their labels conform to the scheme in crickets; the neurons from 9-2a to 10-3a resemble the cricket counterparts most closely. From Schrader et al. (2002), with permission from John Wiley & Sons.

surfaces. Also, at inclinations above 45° the majority of cave crickets face upwards, while below 45° there is no bias in their orienting direction (Kastberger, 1982). Using innovative experimental approaches in free-standing and tethered animals exposed to various stimulation regimes and stimuli combinations, Kastberger (1984) demonstrated that gating of these behaviours is mediated via leg proprioreceptors (Fig. 8A) and modulated by at least two further channels of gain control: the extero-mechanoreceptive and visual inputs. In the following study, these stimuli were shown to influence also the rhythm of abdominal respiratory movements (ARM), which specifically in cave crickets is modulated strongly by posture (Kastberger, 1985). Under most experimental conditions, the cycling of the ARM pacemaker was found retarded in vertical stance and advanced in horizontal stance, suggesting coupling of the ventilatory rhythm to either general state of arousal or locomotor activity. A model for resetting and sensory modulation of ARM was proposed (Kastberger, 1985).

The cercal system of cave crickets for detection of air currents and triggering escape responses differs from other orthopteroids in that the abdominal cerci covered with sensory hairs are oriented perpendicularly and not parallel to the ground. The effect of this difference was studied in *T. neglectus* behaviourally and at the level of cercal giant interneurons (GIs; Schrader, 2000; Schrader et al., 2002). Cave crickets well detect the direction of wind stimuli and jump away from a wind puff (Fig. 8B), but in contrast to crickets they jump directly away from the stimulus without turning their body first; e.g. if stimulated from the front, the animal jumps backwards (Schrader 2000). Five types of wind-sensitive GIs (Schrader et al., 2002; Fig. 8C) and an additional two types of local interneurons (Schrader, 2000) were identified in the terminal abdominal ganglion of the ventral nerve cord. Four dorsal GIs closely resemble their putative cricket homologues morphologically and in directional sensitivity, while the ventral GI differs somewhat from its cricket counterpart in the location of dendritic branches and the responsiveness to horizontal stimuli. The local neurons were suggested to modulate directional sensitivity of the GIs with inhibitory inputs. As no change in directional sensitivity was found in cave crickets at the level of central processing, the study presumed compensatory changes in directional preferences of sensory hairs due to the changed orientation of the cerci (Schrader et al., 2002).

Chemosensory systems and communication

In the absence of long-distance acoustic signals and also useful visual signals for nocturnal and cavernicolous Rhaphidophoridae, these species have been traditionally regarded to strongly rely on chemical communication. Convergently developed hypodermal glands were long known from males of different genera, and in the most elaborated form from *Troglophilus* (Seliškar, 1923). Here, two pairs of large gland sacks are present dorsally in the male abdomen, being filled with a red secretion that is strongly aromatic. Each pair of gland sacks protrudes to the body surface within a dermal bulb between the subsequent abdominal tergites (Fig. 9A$_i$). Almost a century ago, the Slovenian physiologist and speleobiologist Albin Seliškar investigated histology and development of these glands in *T. neglectus* and *T. cavicola* and tried to elucidate

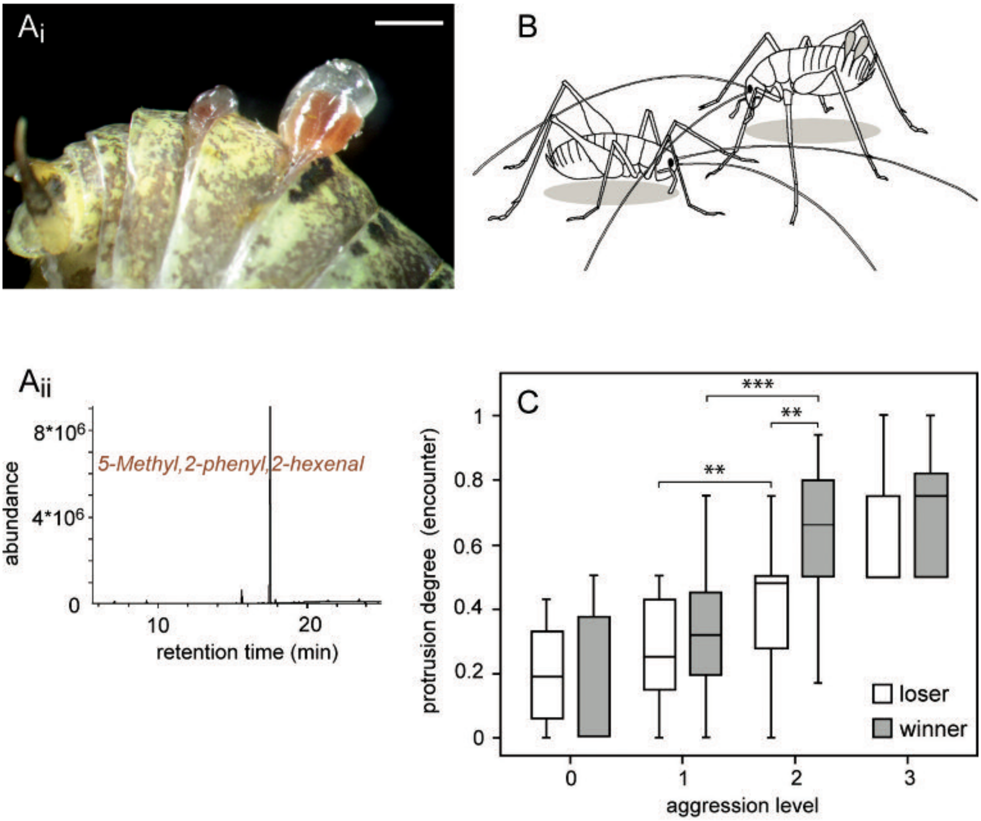

Fig. 9: Scent glands in *T. neglectus*, the released odour and its role in inter-male aggression. **A$_i$)** Protrusion of dermal bulbs, containing gland sacks with red secretion, induced in the intersegmental regions between 5th–7th abdominal segments by slight compression of the male's abdomen. **A$_{ii}$)** Gas chromatogram of the released volatile. Large amounts of 5-Methyl, 2-phenyl, 2-hexenal release was demonstrated from only briefly protruded glands, typical for *T. neglectus* contests. **B)** Drawing reconstruction of a male pair in a contest. The dominant male (right), with an elevated (i.e. aggressive) body posture, protrudes both gland bulbs in the phase just prior to attack. The subordinate male has glands retracted and shows a lowered (submissive) posture just prior to retreat. **C)** Gland protrusion degree (a combined, normalized measure on the frequency-intensity of protrusion) in winners and losers of individual encounters (contests) at different levels of aggressive escalation. The diagram shows the increase of gland protrusion in the aggressive context, its significant increase in both contestants with the occurrence of the elevated body posture (aggression level 2), and its decisive influence for winning/losing a contest at this level of aggressive escalation, just prior to an attack (aggression level 3). The asterisks relate to different degrees of statistical significance (for more details see Stritih and Žunič Kosi, 2017). Adapted from Stritih and Žunič Kosi (2017).

their function by behavioural observations (Seliškar, 1923). He showed that the gland epithelium becomes functional after the final molt, is much more extensive in *T. neglectus* than in *T. cavicola* and protrudes spontaneously to the body surface only in the former species. Making interspecific behavioural comparisons to orthopteroids, like tree crickets and cockroaches, he generally presumed the function of the released odour in attraction and/or sexual stimulation of females (Seliškar, 1923). Much later on, 5-Methyl-2-phenyl-2-hexenal was identified as the major volatile compound from gland secretion in *Troglophilus* (Raspotnig et al., 1998; see also Fig. 9A_{ii}). The compound was offered to larvae and adults of both sexes in a simple experiment that elicited no behavioural response. Our later studies demonstrated the male odour to function in the complex behavioural context of inter-male aggression (Stritih, 2014; Stritih and Žunič Kosi, 2017). By analysing pre-mating behaviour within small male-female groups of *T. neglectus*, we showed that gland protrusion does not necessarily accompany male courtship or pre-mating time, in general, and causes neither female attraction nor influences courtship success (Stritih, 2014). Gland protrusion occurred most frequently during male-male encounters and particularly their aggressive behaviour, thus implying the function of the released odour as an inter-male agonistic signal (Stritih, 2014). In the following study we investigated the details of male contest behaviour and correlated them with the frequency and extent of gland tissue protrusion in each individual as an indication of the amount of released odour (Stritih and Žunič Kosi, 2017; Fig. 9B, C). These correlations suggested the odour to function as an aggressive threat signal that significantly influences contest resolution (Fig. 9C), as well as signals dominance, thereafter. We further manipulated the signal exchange by preventing gland tissue protrusion in the contestants, and analysed the behavioural consequences of different treatments (Stritih and Žunič Kosi, 2017). This approach directly demonstrated that the odour functions as a highly effective threat that prevents maximal contest escalation and decreases the contest-related costs. The study provided the first evidence of olfactory signalling of aggressive intent (motivation) in a terrestrial animal, using a system of eversible scent glands for the instantaneous modulation of odour release along with the changing behavioural context.

Conclusions and outlook

The mechanosensory and chemosensory systems described in *Troglophilus* show a mixture of ancestral and derived characters, not only in comparison to other Ensifera and orthopteroids but also between the two investigated species. While *T. neglectus* and *T. cavicola* appear highly similar in morphology and their general ecological preferences, their sexual behaviour and communication strongly differ. Apparently, these differences reflect a higher level of cavernicolous adaptations in *T. cavicola* related to the reproductive period in comparison to its sympatric species. Studying different aspects of their sensory physiology, sensory anatomy, biophysics and behaviour in a comparative framework markedly increased our understanding of the function and evolution of these systems. The current level of knowledge provides us with a line of further questions to be answered, thus keeping *Troglophilus* as a promising area of future research.

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