Ultrastructure and electrophysiology of thermosensitive sensilla coeloconica in a tropical katydid of the genus *Mecopoda* (Orthoptera, Tettigoniidae)

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In many acoustic insects, mate finding and mate choice are primarily based on acoustic signals. In several species with high-intensity calling songs, such as the studied katydid *Mecopoda* sp., males exhibit an increase in their thoracic temperature during singing, which is linearly correlated with the amount of energy invested in song production. This increased body temperature is used by females as an additional cue to assess the male's quality during mate choice, as has been recently hypothesized ("hot-male" hypothesis). Thermosensory structures would be required to evaluate this cue. In the present study, therefore, we investigated the ultrastructure and physiology of thermosensitive sensilla coeloconica on the antennal flagella of *Mecopoda* sp. using a combination of electron microscopy and electrophysiological recording techniques.

We could identify three distinct types of sensilla coeloconica based on differences in the number and branching pattern of their dendrites. Physiological recordings revealed the innervation by antagonistically responding thermoreceptors (cold and warm) and bimodal hygro-/thermoreceptors (moist or dry) in various combinations. Our findings indicate that *Mecopoda* sp. females are capable of detecting a singing male from distances of at least several centimetres solely by assessing thermal cues.

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acoustically communicating cricket and katydid species revealed that this increase in thoracic temperature is positively correlated in a linear manner with the energy invested during song production (Erregger et al., 2017). Females could use this thermal information for mate choice decisions, for example, to evaluate how much energy has been invested in song production. This potential function of thermal stimuli in close range mate choice scenarios has been termed the “hot-male” hypothesis (Erregger et al., 2017, 2018).

In a first approach to test this hypothesis, Schneider and Römer (2016) investigated the typology and distribution of sensory structures on the antennae of two tropical katydids of the genus Mecopoda. Based on the outer morphology of the cuticular apparatuses, the authors could identify nine different types of sensilla. Two of these, the basicicon sensillum 2 (ba2) and the coeloicon sensillum (co), proved to be good candidates for a thermoreceptive function (Schneider and Römer, 2016). Preliminary electrophysiological recordings of co in Mecopoda sp. additionally revealed the activity of two antagonistically responding thermoreceptors in the form of a cold and a warm cell (L.M. Zopf and H. Römer, unpublished results). The results of several morphological and physiological studies revealed an innervation by a physiological triad consisting of two hygroreceptors (moist and dry) and one thermoreceptor bulb (cold) of the poreless sensilla with an inflexible socket (Orthoptera: Altner et al., 1981; Itoh et al., 1984; Nishikawa et al., 1985; Phasmatoidea: Altner et al., 1978; Tichy, 1979; Heteroptera: Bernard, 1974). The same innervation pattern with two antagonistically hygro- and one cold-receptor has also been found in other poreless sensilla with inflexible sockets (np-is sensilla), but these have been termed differently due to their outer morphology as either dome-shaped sensilla (Coleoptera: Nurme et al., 2015; Nurme et al., 2018), sensillum capitulum (Blattodea: Tominaga and Yokohari, 1982; Yokohari, 1981), sensillum styloconicum (Lepidoptera: Steinbrecht, 1989), or sensillum coeloapatapum (Hymenoptera: Yokohari et al., 1982; Yokohari, 1983). In contrast, reports on sensilla with two antagonistically acting thermoreceptors (cold and warm), as indicated for Mecopoda sp., have been mentioned much more rarely in the literature. These include the co of the mosquito Aedes aegypti (Davis and Sokolove, 1975; McIver, 1973) and of the true bug Rhodius prolixus (McIver and Siemicki, 1985; Zopf et al., 2014), as well as the trichoid hairs of R. prolixus (Zopf et al., 2014), the tick Amblyomma variigatum (Hess and Loftus, 1984; Hess and Vimant, 1983) and the cave beetle larvae Speophyes lucidulus (Corbière-Tichané, 1971; Corbière-Tichané and Loftus, 1983; Loftus and Corbière-Tichané, 1981). In all of these species, the perception of thermal stimuli seems to be of particular importance. The first three mentioned are obligate blood feeders and strongly depend on thermal stimuli to find a suitable host or biting area, without which the successful reproduction and survival of the species is not possible (Bodin et al., 2009; Davey, 1965; Klowden, 1995; Olivier, 1989). Larvae of the cave beetle, on the other hand, live in dark caves and are devoid of eyes. It is assumed that the thermal sense in this species may serve for orientation or retention of the animals within the caves and, therefore, may compensate for their lack of a visual system to a certain degree (Corbière-Tichané and Loftus, 1983).

Apart from these rather specialised functions of thermosensation, it can be assumed that probably all insects are capable of perceiving thermal and hygri stimuli, as these are crucial for an effective thermoregulation behaviour and maintenance of a stable water balance to avoid overheating and desiccation (Edney, 2012; Heinrich, 1995). In the present study, we investigated the ultrastructure and physiological responses of antennal co in the katydid Mecopoda sp. to thermal and humidity stimuli, using a combination of electron microscopy and extracellular electrophysiological recordings.

2. Material and methods

2.1. Animals

Experiments were performed with adult individuals of the trilling katydid species described by Korsunovskaya (2008) as Mecopoda sp. 4. The taxonomy of the genus Mecopoda is still unresolved. Several sibling species are morphologically similar but have distinctly different calling song patterns (Nityananda and Balakrishnan, 2006). Insects included in the present study were taken from a laboratory breed maintained at the Institute of Zoology in Graz, which was originally established from individuals collected in a tropical rainforest in Malaysia in 2010 and 2011. Animals were kept at 27 °C and 70% relative humidity with a 12h:12h light:dark cycle. Fish food, oat flakes, lettuce, apple pieces and water gel were given ad libitum.

2.2. Distribution of sensilla coeloconica (co) on the antennal flagella

After conducting thorough initial analyses of several specimens by light microscopy and, subsequently, scanning electron microscopy, it was also possible to identify co on the antennal flagella with high accuracy merely by using light microscopy. We used a Wild MSA stereomicroscope (Wild Heerbrugg, Switzerland) operated at magnifications of 100× and 200× and a Leica CLS 150 LED with flexible gooseneck as light source, which was positioned on one side underneath the specimen holder (perpendicular to the longitudinal axis of the antenna) to obtain a more diffuse light source and fewer reflections at the antennal surface. To analyse the distributional pattern of co, animals were decapitated after being anesthetized with ethyl chloride. Antennae, still attached to the head capsule, were mounted on a custom-made Plexiglas (poly-methyl methacrylate) holder, which allowed for the continuous rotation of the antennae around the longitudinal axis. In order to not miss any sensilla, each antenna was analysed from six different viewing points with a strong degree of overlap: the ventral and dorsal sides were positioned upward and, from each of these positions, they were rotated −60° and +60° around the longitudinal axis of the antenna. The number and relative positions of co on the flagellar segments were recorded from each of the six viewing points, starting from the first (most proximal) through to the last (most distal) segment, using a voice recorder. To avoid obtaining false positive results, only those sensilla were counted that had been identified from at least two different viewing points during the subsequent analyses. We analysed three and four antennae of individual female and male Mecopoda sp., respectively (Total n = 7).

2.3. Specimen preparation and transmission electron microscopy

Animals were anesthetized with ethyl chloride and mounted on a Plexiglas holder. Their antennae were positioned lateral side upwards and reversibly fixed with small stripes of adhesive tape. Afterwards, the position of antennae was finely adjusted under light-microscopic control (Wild MSA stereomicroscope, Wild Heerbrugg, Switzerland) in such a way that as many co as possible faced exactly upwards. Small cactus needles (Opuntia sp.) were then inserted into the upper side of the flagellar segments that bore at least one perfectly oriented co, maintaining a sufficient distance to ensure that the sensilla were not injured. The antennae were then tilted around their longitudinal axis to 90°. Marked flagellar segments were fixed at both ends with a small drop of water-based white-out fluid (Tipp–Ex ecolutions, Bic, France). After this fluid dried, the flagellar segments were covered with a large dot of iced 0.05 M cacodylate buffer containing 3% glutardialdehyde (Carl Roth, Germany). About one-third of each segment on the side opposite
the marked sensilla was carefully removed using fine microscalpels. This procedure facilitated the infiltration of the fixative and resulted in a considerably better preservation of the tissue. Afterwards, the flagellar segments were carefully excised and immediately transferred to a fresh fixative solution (3% glutaraldehyde in 0.05 M cacodylate buffer), where they remained overnight at 4°C. After being subjected to 2 h of post-fixation with 1.5% OsO₄ in the same buffer and rinsed three times with fresh buffer solution, specimens were dehydrated using a graded ethanol series and embedded in Epon 812, which had a mixing ratio of 5:5 of mixtures A and B according to Lüft (1961). Semi-thin sections with a thickness of 0.5–1 μm were cut with a histo-diamond knife (DIATOME, USA) using a Leica 2065 Supercut microtome (Leica Microsystems, Germany). Ultra-thin sections with a thickness of about 70 nm were cut with 45° and 35° ultra-diamond knives (DIATOME, USA) using a Leica Ultracut UCT microtome (Leica Microsystems, Germany). Utilization of 35° knives resulted in considerably reduced compression and fewer cracks, which had prevented the visualization of the structures when the ultra 45° diamond knife was used. Semi-thin sections were stained with a 0.1% toluidine/borax solution and examined using an Olympus BH2 light microscope (Olympus, Japan). Ultra-thin sections were double-stained with 300 ppm platinum blue for 15 min and 3% lead citrate for 7 min, then examined with a FEI Tecnai G2 transmission electron microscope (FEI, Thermo Fisher Scientific, USA) using an accelerating voltage of 120 kV. We prepared and analysed consecutive ultra-thin series of seven co from three individual, female Mecopoda sp..

2.4. Scanning electron microscopy

After marking the samples with cactus needles as described above in section 2.3, Specimen preparation and transmission electron microscopy, antennae were removed from the head capsule and cut into 1.5–2.0 cm long pieces, each bearing at least one cactus needle for orientation. Samples were dehydrated using a graded series of aqueous ethanol solutions and subsequently sonicated in a 1:1 mixture of chloroform and ethanol for several minutes. After drying, specimens were mounted on aluminium stubs with adhesive conductive carbon tape (Leit-C tabs, Plano GmbH, Germany) and small drops of liquid carbon (Leit-C, Plano GmbH, Germany) were applied to both ends. The samples were sputter coated with gold/palladium for 60 s at 40 mA using a Bal-Tec SCD 500 sputter coater (Bal-Tec AG, Switzerland). Samples were observed with a Zeiss DSM 950 scanning electron microscope (Carl-Zeiss, Germany) using an accelerating voltage of 15 kV.

2.5. Extracellular electrophysiological recordings

2.5.1. Temperature and humidity stimulation set-ups

Temperature stimulation via convective heat was conducted using a procedure similar to that used by Nagel and Kleineidam (2015). Filtered and dried air (1.4 g/m³ absolute humidity) was split into two separate airstreams that were controlled by proportional flow meters (SLPM 35831, Analyt-MTC, Germany) using an instrument control from LabView (LabView 2011, National Instruments, USA). The separated airstreams were heated or cooled down, respectively, with a water bath-based, counter flow heat exchange system (workshop built, University of Konstanz) and were fused again close to the recording site. The nozzle of the fused air stream was directed onto the recording site of the antenna at a distance of 2–3 cm. Adjusting the ratio of the two airstreams allowed the application of different temperatures and temperature-changing rates at a constant flow rate of 2 l/min. Maximum temperature-changing rates ranged between ±0.014 and ±0.67 °C/s.

For stimulation, we used temperature oscillations with a constant frequency of 0.025 Hz. Stimulation started at temperatures between 25.5 and 26.3 °C of the mixed air stream (50:50 ratio of cold:warm air) beginning with a cold-stimulus for 10 s at a given temperature-changing rate followed by alternating phases of temperature increase and decrease with durations of 20 s at the same temperature-changing rate but with different directions. Therefore, a maximum temperature range of 19–33 °C was used during oscillations. The stimulation always followed an adaptation time for at least 3 min to the temperature of the mixed air stream with a ratio of 50:50 of cold:warm air.

Humidity stimulation was conducted using a procedure similar to that above except that the counter flow heat exchange system, which heated or cooled down the two separated airstreams carrying dried air, was bypassed. Instead, one of the air streams was saturated with water by channeling it through a washing bottle. By adjusting the ratio of the two airstreams (dry and moist air), different values of relative humidity (6–94% RH) and relative humidity-changing rates at a constant flow rate of 2 l/min and at constant temperatures between 25.1 and 26.2 °C were applied. For humidity stimulation, we used oscillations with a frequency of 0.025 Hz and maximum changing rates of relative humidity between ±0.08%/s and ±4.1%/s. As for temperature stimulation, an adaptation time of at least 3 min to the mixed air stream with a ratio of 50:50 of moist:dry air preceded humidity stimulation.

For infrared stimulation, we used a small Peltier element (Tru Components, Conrad Business Supplies, Germany) positioned at a distance of 2.6 cm from the sensillum site. Infrared oscillations were generated by manually changing the polarity of the Peltier element, which was fed by a 4.5 V battery. This resulted in cyclic heating/cooling of the Peltier element. In order to obtain a defined emissivity of the infrared source, the surface of the Peltier element facing the sample was covered with an emission adhesive tape (Testo AG, Germany, Model No.: 05540051; emissivity (ε) = 0.95). Temperatures at the sensillum site and of the infrared source were measured with T/C-thermocouples. The stimulus intensity (I) was calculated on basis of the Stefan–Boltzmann law, modified by De Cock Buning (1983), using the formula:

\[ I = \frac{\varepsilon \times \sigma \times (T_1^4 - T_2^4) \times A_{\text{rad}}}{\pi r^2 \times \eta} \]  

in which ε is the emissivity of the emission adhesive tape coating the infrared source (ε = 0.95); \( \sigma \) is the Stefan–Boltzmann constant with 5.67 × 10⁻⁸ W m⁻² K⁻⁴; \( T_2 \) is the surface temperature of the infrared source; \( T_1 \) is the temperature at the sensillum site; \( A_{\text{rad}} \) is the radiating area of the infrared source (2.56 × 10⁻⁴ m²) and \( r \) is the distance of the infrared source to the antenna. During infrared stimulation, a continuous air stream with constant humidity (1.4 g/m³) and temperature (26.3 °C) at a constant flow velocity of 2 l/min was blown over the antenna at the recording site to exclude the effect of convective heat.

2.5.2. Preparation and recording

Individuals of Mecopoda sp. were anesthetized with ethyl chloride and immobilized on a Plexiglas holder with paraffin. The flagellum of the antenna was mounted under visual control (Leica S8AP0, Leica Microsystems, Germany) with narrow stripes of adhesive tape and water-based, white-out correction fluid (Tipp-Ex ecollutions, Bic, France), exposing the dorsolateral side upwards. The distal end of the flagellum was submerged in a drop of conductive gel (Hydro Sensitiv Gel, Ritex, Germany) and the most distal segments were then cut off with a sharp razor blade. As a reference electrode, a chlorinated silver wire was placed in the...
same drop. The recording electrode was an electrolytically sharpened tungsten wire, which was inserted into the base of the cuticular apparatus just deep enough to make an electrical contact. The precise positioning of the recording electrode was controlled by a digital micromanipulator (Nanocontrol INC40, Kleindiek, Germany) under visual examination using a microscope (Axio Examiner A1, Zeiss, Germany). Recordings were band-pass filtered (10 Hz–2 kHz), amplified 100× (BA-03× amplifier, npi, Germany) and digitally filtered (Humbug, Quest Scientific, Canada) to reduce electrical noise. Signals of sensory neurons were sampled at a rate of 25 kHz.

Temperature was recorded with PFA-insulated, T/C thermocouples (STC-TT-RTI1, Omega Engineering, US and Canada) that were placed about 2 mm behind the recording site and, for infrared stimulation, also on the surface of the Peltier element, directly under the emission-adhesive tape. The thermocouples were connected to a thermocouple input module (NI9214, National Instruments, USA), and signals were recorded at a sampling rate of 4 Hz, resulting in an accuracy of temperature measurements of 0.01 °C. Humidity was recorded with an integrated circuit humidity sensor (Honeywell International Inc., model HIH 4000-002) at a sampling rate of 25 kHz. The humidity sensor was interposed into the air flow after fusing the separated moist- and dry-air streams. In total we analysed electrophysiological responses of 14 sensilla, of which eight and six were from five individual females and three individual males, respectively.

2.5.3. Data analysis

Spike recordings were analysed with Spike2 software (Spike2, v5.21, Cambridge Electronic Design, UK). Spikes of different sensory neurons could be discriminated based on their shape, amplitude and their response characteristics to oscillating changes in the stimulus temperature and humidity. Depending on the type of stimulation, changes in spike amplitude and especially in the ratio of amplitudes of different neurons observed during single recordings sometimes made spike discrimination difficult. Therefore, spike amplitude and waveform also had to be examined by visual inspection from impulse to impulse. If not otherwise stated, all calculations on neuronal activity and corresponding stimuli were done in 1-s windows. As a measure of response magnitude, the peak firing rate after stimulus onset was taken. The rate of change in temperature [°C/s] and relative humidity [%/s] was determined by calculating the maximum slope of the stimulus amplitude. All further analyses were done using MS Excel.

3. Results

3.1. Outer morphology and distribution of sensilla coeloconica (co) on the antennal flagellum

Co in Mecopoda sp. are a porous, peg-in-pit sensilla with a small peg on an inflexible socket within a chamber that is embedded in the antennal cuticle. The peg inside the chamber is exposed to the surrounding air via a small aperture (Fig. 1). The sizes of the chambers and the apertures were highly variable and depended on their position on the antenna. Both parameters gradually increased from proximal to distal flagellomeres (Fig. 2). Except for a clogged molting pore at its apical tip, the surface of the peg was smooth and showed no indications of wall pores (Fig. 1c). Based on their outer morphology, we could not identify any parameters that would allow for a discrimination of different types of co, nor could we find any sexual dimorphism. A comprehensive description of different morphometric parameters of co measured in Mecopoda sp. can be found in Schneider and Römer (2016).

Fig. 2. Sizes of pits (blue diamonds) and pit apertures (red squares) of co in relation to their position on the antennal flagellum, measured on a single individual. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 1. SEM-micrographs showing the outer morphology of sensilla coeloconica (co) on the antennal flagellum of Mecopoda sp. from side view (a) and top view (b). The co consist of a small, straight peg that is perpendicular to the floor of a pit sunken in the cuticle. A small aperture exposes the peg inside the pit to the surrounding air. c) Higher magnification image of the peg inside the pit; except for a clogged molting pore (arrowhead) at its apical tip, the peg surface is smooth and devoid of wall pores. Scale bars: a) and b) 5 μm; c) 1 μm.
Table 1
Numbers and relative positions of co on the antennal flagella, measured on seven individual specimens. Calculated values are given as mean ± standard deviation.

| Specimen | 1    | 2    | 3    | 4    | 5    | 6    | 7    | Mean (abs.) | Mean (rel.) [%] |
|----------|------|------|------|------|------|------|------|-------------|----------------|}
| Number of peg in pits |          |      |      |      |      |      |      |             |                |
| Lateral  | 105   | 91   | 23   | 50   | 54   | 66   | 67   | 65.1 ± 27.1 | 79.3 ± 15.2 |
| Dorsal   | 9     | 0    | 3    | 0    | 2    | 3    | 64   | 11.6 ± 23.3 | 10.1 ± 16.4 |
| Ventral  | 18    | 18   | 2    | 2    | 4    | 13   | 1    | 8.3 ± 7.8   | 9.0 ± 6.0   |
| Medial   | 0     | 0    | 0    | 0    | 0    | 6    | 6    | 1.7 ± 2.9   | 1.6 ± 2.8   |
| Total    | 132   | 109  | 28   | 52   | 60   | 88   | 138  | 86.7 ± 41.9 |                |
| At occurrence on single segment | 1.3 ± 0.5 | 1.4 ± 0.6 | 1.1 ± 0.3 | 1.1 ± 0.2 | 1.2 ± 0.4 | 1.3 ± 0.6 | 1.3 ± 0.5 | 1.2 ± 0.1 |                |
| No. of flagellar segments |          |      |      |      |      |      |      |             |                |
| Total    | 194   | 191  | 132  | 165  | 187  | 174  | 199  | 177 ± 23    |                |
| With peg in pits | 104   | 80   | 25   | 49   | 52   | 68   | 103  | 69 ± 29     | 37.5 ± 12.8  |

Fig. 3. Schematic drawing of a Type I co, reconstructed on the basis of ultrathin sections. Lettered dashed lines indicate positions of transverse sections shown in Fig. 4. Type I co are innervated by three dendrites (numbered in Arabic numerals), one of which has a lamellated dendritic outer segment. All three dendrites distally innervate the peg, tightly filling its lumen. Cell bodies, axons and inner segments of the dendrites are highly similar to one another. Each sensillum is associated with three types of enveloping cells. Abbreviations: cu = cuticle; ds = dendritic sheath; ec = epidermal cell; eds = electron dense structure; irlc = inner receptor lymph cavity; orlc = outer receptor lymph cavity; the = thecogen cell; tor = tormogen cell; tri = trichogen cell. Scale bar: 5 μm.
Fig. 4. TEM-micrographs of transversal ultrathin sections through a Type I co. a) At 1.0 μm proximal to the tip of the peg, two dendrites, one of which is lamellated, tightly fill the peg lumen. Four apical branches of the lamellated dendrite (d1) are discernible. Scale bar: 200 nm. b) At 1.7 μm proximal to the tip of the peg, a second unbranched dendrite (d3) becomes visible. At the inner surface of the peg wall, a separate layer of higher electron density (arrowhead in subfigures a) and b)), which is in continuity with the dendritic sheath (ds), can be discerned. Scale bar: 200 nm. c) Section 5.8 μm proximal to the tip of the peg; three dendrites, one of which is clearly folded, are enclosed by a thick ds (arrow). A large electron dense structure (eds) is tightly apposed to the ds. Scale bar: 400 nm. d) At 9.8 μm proximal to the tip of the peg, the ds, still thick, is enveloped by the trichogen (tri) and the tormogen cell (tor). Note that the outer receptor lymph cavity (orlc) has a medium-electron-dense appearance. Scale bar: 400 nm e) At 13.7 μm proximal to the tip of the peg, the thickness of the ds (arrow) is significantly reduced. The folding of the lamellated dendrite (d1) is considerably reduced. Scale bar: 400 nm. f) At 21.3 μm proximal to the tip of the peg, the continuous ds is no longer present. Dendrites are now enveloped by the thecogen cell (the); on its inner side, bundles of parallel oriented microtubules (mt) are visible. Scale bar: 500 nm. g) At 24.2 μm proximal to tip of the peg, the two unbranched dendrites (d2, d3) already show 9 × 2 configurations of their microtubules, indicating the transition from the outer to the inner dendritic segment (DIS). Note the electron-dense appearance of the inner-receptor lymph cavity (irlc). Scale bar: 400 nm. h) Section 24.7 μm proximal to the tip of the peg at the level of the ciliary constriction; Scale bar: 400 nm. i) Section 25.5 μm proximal to the tip of the peg at the level of the DIS; proximal basal bodies of dendrites d1 and d2 are discernible. Scale bar: 400 nm. Abbreviations: d1 - d3 – dendrites 1–3; eds – electron dense structure; irlc – inner receptor lymph cavity; mt – bundles of microtubules; orlc – outer receptor lymph cavity; the – thecogen cell; tor – tormogen cell; tri – trichogen cell.
Co were distributed over the whole length of the antennal flagellum in a similar pattern for both sexes. We found total numbers of co ranging between 28 and 138 per single flagellum with one to four on a given flagellomere (Table 1). Taking into account the whole antennae, these numbers correspond to mean values of 0.21–0.69 co per single flagellomere. Regarding the average numbers of co, we could not find any significant differences between both sexes (Mann–Whitney U test; p-value = 0.629). On average, more than 79% of co were located on the lateral and less than 2% on the medial side of the flagellum (Table 1).

3.2. Ultrastructure of sensilla coeloconica (co)

Based on the results of ultrastructural analyses, we could identify three different types of co (I–III) on the antennal flagellum of Mecopoda sp.. Of a total of seven sensilla, we could classify three each as type I and type II and one as type III. Besides the number of sensory cells, differences between these types could especially be identified at the level of their dendritic outer segments (DOS) regarding 1) the branching pattern of dendrites and 2) the thickness of the dendritic sheath (ds).

Type I co were characterized by the presence of three sensory cells, one of which had a lamellated DOS (Figs. 3, 4, and 7). The remaining two dendrites were unbranched. Type II and type III co possessed three and two neurons with unbranched DOS, respectively (Figs. 5–7). In the particular case of the type III co, the lumen of the ds provided enough space for a third DOS, although only two sensory cells were present. This additional space was filled by an electron-dense, granular material (Fig. 6), while all other structural features strongly resembled those of the type II co. We, therefore, assume that a reduction in sensory cell number has taken place in this specific type.

The general sensillum structure regarding the outer cuticular apparatus, the perikarya of the sensory cells, as well as the arrangement of enveloping cells were quite similar in all types of co (cf. Fig. 3). The lateral wall of the peg is smooth and not pierced by pores (Figs. 4–6). Only at its apical tip, we could identify a slightly invaginated pore. It was always clogged with electron-dense material and measured about 0.3 μm in diameter (Fig. 6a). Its appearance strongly resembled that of a typical molting pore. The socket of the peg was clearly inflexible, since we could not identify any structures that would facilitate the deflection of the peg, such as a thinner cuticle surrounding its base. The lumen of the peg is completely filled by the apical portions of the DOS with no space left around them for a lymph cavity (Figs. 3–7). In all three types of co, the dendrites proceed with their DOS into the peg, terminating close to its tip (Fig. 7). On the inner surface of the peg, which encloses the DOS, a separate cuticular layer is distinguishable from the outer cuticular parts by its high degree of electron density (Figs. 4a, b, 5a and 6b). This layer measured between 70 and 110 nm and appeared to be continuous with the ds below the peg. The ds was on average 17.2 ± 6.2 μm (mean ± std.-dev.; n = 7) long and extended to the distal part of a trichogen cell near the inner-receptor lymph cavity (Fig. 3). The lumen of the ds was completely filled with the DOS that were tightly applied to its inner surface (Figs. 4a, b, 5a and 6b). The ds often displayed an irregular and bulged outline and was greatly thickened (Figs. 4c, d, 5c and 6d), especially in the region between 1 and 4.5 μm beneath the peg (Fig. 7). There, the wall of the ds exhibited a mean maximum thickness of 709 ± 124 nm (mean ± std.-dev.; n = 3), 406 ± 7 nm (mean ± std.-dev.; n = 3) and 366 nm (n = 1) in type I, type II and type III co, respectively. All types of co are associated with the typical set of three enveloping cell types, a thecogen, a trichogen and a tormogen cell that displayed the common cytological features. The outer receptor lymph cavity is restricted to the space between the ds and the surface of the trichogen and the tormogen cell (Fig. 3). Its lumen is filled with material of medium electron density (Figs. 4d, 5b, c and 6c).

One specific feature found in all samples was a distinctly electron-dense structure located at the transition zone between the outer receptor lymph cavity and the trichogen cell (Fig. 3). This structure had an almost spherical shape with an electron density that was comparable to that of the ds or even denser (Figs. 4c, 5b, d, and 6c). The most conspicuous feature was its enormous size with a maximum diameter of 3.1 ± 0.7 μm (mean ± std.-dev.; n = 6). This specific, electron-dense structure bordered directly on the ds and was sometimes accompanied by further, smaller structures of comparable electron density (Fig. 6c).

An inner-receptor lymph cavity of regular size could be found in the region of the ciliary constriction between the thinner dendrites and the thecogen cell by which they are encapsulated (Fig. 3). As in the outer receptor lymph cavity described above, the inner lymph cavity was also filled evenly with electron-dense material (Figs. 4g, 5f and 6g). On the inner side of the thecogen cell, surrounding the inner lymph space, bundles of parallel-oriented microtubules were abundant (Figs. 4f–h, 6g). These bundles extended distally from the termination of the ds down to the distal part of the dendritic inner segments (DIS). Within a given sensillum, the number of DOS was always consistent with the number of basal bodies, DIS and sensory cells. The latter did not show any cytological peculiarities.

3.3. Physiological response characteristics to temperature and humidity stimulation

Based on the neuronal response characteristics to temperature and humidity oscillations, we could identify four different types of receptor cells: antagonistically responding thermoreceptors (cold, warm) and bimodal hygro-/thermoreceptors (moist/warm, dry/warm). The exact determination of the individual receptor cells and their respective modalities within a single sensillum proved to be difficult, especially due to the bimodality of the hygro-/thermoreceptors. A thorough comparison of several recordings with temperature and humidity stimuli of different amplitudes was required. Spike amplitudes and waveforms of different receptor cells were often quite similar and also changed over the course of time during single and between different recordings. Moreover, the working ranges of the temperature receptors were especially found to be highly variable and often extended beyond the range of temperatures tested (19–33 °C). Therefore, an unambiguous determination of the sensory modality or a quantitative analysis of the response characteristics was not possible in all investigated co.

Regardless of their modality, all receptor cells responded in a phasic-tonic manner. The cold cells responded to increasing and
responses to changes in relative humidity, but also to temperature.

The bimodal hygro-/thermoreceptors exhibited the strongest responses to changes in relative humidity, but also to temperature changes, albeit not to the same extent as the monomodal thermoreceptors described above (Fig. 8). The moist/warm cells responded to increases and decreases in the relative humidity and in the temperature with increases and decreases in impulse frequency, respectively (Fig. 8b, b’). The dry/warm cells responded in a similar manner to thermal stimuli in that the impulse frequency increased and decreased in response to increasing and decreasing temperatures, respectively (Fig. 8c). Their response to changes in relative humidity, however, were the opposite, whereby increases and decreases in the relative humidity evoked responses of a decreasing or increasing firing rate of the neuron, respectively (Fig. 8c’). Fig. 8 provides an overview of the qualitative response properties of the different receptor cells described above.

The dose-response curves depicted in Fig. 10 demonstrate the quantitative response properties of three individual cold and warm cells as well as of two dry/warm and moist/warm cells. We plotted the change of the neuronal activity in response to the corresponding stimuli at different changing rates. By extrapolating the corresponding logarithmic regression lines, we determined the sensitivity thresholds of the receptor cells, namely, the rate of stimulus change that elicits an increase in the neuronal response of 1 imp/s over the resting activity. In the case of the cold cells, threshold sensitivity was between −0.008 and −0.051 °C/s and, for the warm cells, on the same order between +0.006 and +0.011 °C/s. The defined threshold for the dry/warm cells and the moist/warm cells was found with a rate of change in relative humidity between −0.071 and −0.105%/s and +0.008 to +0.193%/s, respectively (Fig. 10).

3.4. Physiological combinations of thermo- and hygroreceptors in sensilla coeloconica (co)

In the electrophysiological experiments, up to a maximum of three receptor cells were recorded from single co. Out of a total of 14 sensilla that have been analysed electrophysiologically, we could record signals of three individual receptor cells each in 10 co (Table 2). In four of these ten sensilla, we could unambiguously identify the adequate sensory modality of all three receptor cells. Three of these housed two antagonistically responding thermoreceptors (cold, warm) and a single bimodal hygro-/thermoreceptor (moist/warm). The fourth co comprised one cold and two warm receptors. In the other six sensilla housing three receptor cells, we could unambiguously identify only two cells each. The remaining third cell responded only irregularly and ambiguously in single recordings to thermal stimuli at the upper or lower end of the tested temperature range. Since changes in relative humidity over a range of 6–94% could not elicit any change in response, we assume a thermoreceptive modality for these third cells with a working range beyond the temperatures tested. Under this assumption, all ten co with three receptor cells can be allocated to two distinct, physiological combinations. The first combination comprises two thermoreceptors and one bimodal hygro-/thermoreceptor (6 of 10 co) and the second combination, three thermoreceptors (4 of 10 co). All sensilla possessed at least one cold receptor and, if a hygro-/thermoreceptor was present at all, this could be either a moist/warm or a dry/warm cell (Table 2). The combination of two hygroreceptors

Fig. 7. Schematic drawings of the dendritic outer segments (DOS) of the three different morphological types of co, reconstructed on the basis of ultrathin sections. Several structural components like enveloping cells, receptor lymph cavities and electron-dense structures were omitted. Main differences between the various types of sensilla were found in the number of sensory cells, the branching pattern of their DOS and in the thickness of the dendritic sheath (ds). Maximum thicknesses of the ds were found within the regions marked by dashed lines (i.e. between 4.0 μm and 7.4 μm proximal to the tip of the peg). Type I sensilla are characterized by the thickest ds and the presence of three dendrites, one of which is lamellated. Type II and type III sensilla consist of three and two unbranched dendrites, respectively. Although only two sensory cells were present in the type III co, the lumen of the ds provided enough space for a third DOS. The additional space was filled by granular material (dark grey compartment marked with an asterisk), probably indicating the reduction of a previously existing, third sensory cell. Scale bar: 2 μm.

Fig. 6. TEM-micrographs of transversal ultrathin sections through a Type III co. a) A slight invagination at the very tip of the peg (arrow) indicates the position of a molting pore that is clogged with electron-dense material (asterisk). Scale bar: 200 nm. b) At 0.8 μm proximal to the tip of the peg, two unbranched dendrites (d1, d2) filling the lumen of the peg are visible. Arrowhead marks a separate layer of electron-dense cuticle of the peg wall surrounding the dendrites. Scale bar: 400 nm. c) Section 5.2 μm below the tip of the peg, showing the outer-receptor lymph cavity (orc) and a roundish, electron-dense structure (ed). Note the irregular shape of the thick dendritic sheath (ds; arrow) that encloses two dendrites at the level of their dendritic outer segments (DOS) together with a distinct region filled with granular material (asterisk), which probably represents the remnant of a reduced third dendrite. Scale bar: 1 μm. d) Same section as in b), but at higher magnification. Scale bar: 200 nm. e) At 10.4 μm proximal to the tip of the peg, the ds (arrow) has a rather smooth and regular shape and is significantly reduced in thickness. Scale bar: 400 nm. f) At 18.8 μm below the tip of the peg, the ds begins to dissolve. The two dendrites, still at the level of the DOS, are enveloped by the thecogen cell (tc). Scale bar: 500 nm. g) Ciliary constriction at 21.6 μm proximal to the tip of the peg. The distal (dhb) and proximal basal body (pbb) as well as the ciliary rootlets (cr) of one dendrite are clearly discernible. Dendrites are accompanied by bundles of parallel oriented microtubules (mt). Due to the strong bending of the dendrites below the antennal cuticle in the proximal direction of the antenna, the dendrites are cut longitudinally at this position. Scale bar: 400 nm.
Fig. 8. Physiological responses of receptor cells in co to temperature (convective heat) (a–c) and humidity oscillations (a’–c’). Temperature stimulation was performed at constant absolute humidity of 1.4 g/m³ and humidity stimulation at constant temperatures between 25.1 and 26.2 °C. Images at the top schematically show the course of temperature and humidity stimuli over time, starting at approximately 25 °C and 50% relative humidity (RH), respectively. Stimulus amplitude is given as the absolute difference between maximum and minimum values ($dT$ for temperature and $dRH$ for relative humidity). In the lettered subfigures (a–c, a’–c’), upper traces show the extracted spike trains of the receptor cells and lower traces, their spike frequency with a bin size of 1 s, respectively. a) Responses of a cold (light grey) and a warm cell (dark grey) within the same sensillum to oscillating temperatures with $dT = 2.8$ °C and a’) to oscillating relative humidity with $dRH = 24\%$; inset shows an enlarged view of a 1-s window of the recording. b) Response of a moist-cell to temperature ($dT = 5.9$ °C) and b’) humidity stimulation ($dRH = 22\%$). c) Response of a dry-cell to temperature ($dT = 3.2$ °C) and c’) humidity stimulation ($dRH = 24\%$); inset gives an enlarged view of a 0.5-s window of the recording.
within a single sensillum was never observed in any of the tested co.
Table 2 provides an overview of the number and primary sensory modalities of the receptor cells in the investigated co.

4. Discussion

4.1. Structure-function relationship

The co in Mecopoda differ considerably from common np-is sensilla that have been described in the literature, especially regarding the following structural features: the DOS of all existing dendrites (including the lamellated dendrites) clearly extend into the peg distally. Moreover, we could also identify one type of sensillum (Type II), which houses three unbranched dendrites. In contrast, most of the previously described np-is sensilla have been characterized by the presence of at least three dendrites, two of which were described as unbranched and extending into the lumen of the peg and the third one, lamellated and ending beneath the peg (Altner et al., 1978, 1981; Corbière-Tichane, 1971; McIver, 1973; McIver and Siemicki, 1985; Nurme et al., 2015; Steinbrecht, 1998). Based on these structural differences in combination with the electrophysiological responses of two hygro- and one thermoreceptor (cold), it has been proposed that the lamellated dendrite refers to the cold cell (Altner et al., 1983; Corbière-Tichane and Loftus, 1983; McIver, 1973; Yokohari, 1981). Applying the same rationale on the basis of the present data, however, would lead us to the opposite conclusion, namely, that the lamellated dendrite refers to the hygroreceptor, for the following reasons: 1) all co in Mecopoda sp., also those exhibiting only unbranched dendrites, possessed at least one cold receptor and 2) we could identify both a maximum of one lamellated dendrite morphologically, as well as a maximum of one hygroreceptive unit in the electrophysiological recordings. Since the degree of branching/lamellation of the DOS is proportional to the dendritic membrane area, which may determine the number of membrane-bound ion channels, it seems more reasonable to propose a correlation between the degree of branching and the sensitivity of the receptor, which is also corroborated by the findings of Ehn and Tichy (1996).

Many of the characteristic structural features we identified in the co of Mecopoda sp. have also been found previously in other thermo-/hygrosensitive np-is sensilla. In particular, this includes 1) the location of sensilla, 2) the tight filling of the pegs by the DOS of the dendrites, 3) a thick ds that seems to be continuous with the inner peg wall, 4) electron-dense structures in the region of the

Fig. 9. Neuronal responses of two antagonistically responding temperature receptors within the same sensillum to infrared stimulation. a) Infrared radiation intensity of the stimulus measured at the sensillum site (calculated on the basis of the Stefan-Boltzmann-Law). Infrared stimulation was broadcasted via heating/cooling of a 1.6 x 1.6 cm Peltier element at a distance of 2.6 cm to the sensillum site. b) Temperature measured at the sensillum site during infrared stimulation. Neuronal responses of a cold (light grey) and a warm cell (dark grey) shown as extracted spike train (c) and as spike frequencies with a bin size of 1 s (d, e).
outer-receptor lymph cavity, and 5) the abundance of cytoskeletal elements (i.e. longitudinally oriented microtubules) within the thecogen cell. All these structural characteristics have been discussed previously as indications for hygroreceptors in np-ns sensilla (Altner and Loftus, 1985). It has been hypothesized that hygroreceptors are primarily mechanosensitive and, thus, stimulated by the mechanical deformation of their dendrites through the hygroscopically induced swelling of the peg wall or other cuticular structures (Altner et al., 1978, 1983; Tominaga and Yokohari, 1982; Yokohari, 1978, 1981). Both the position of the peg, which is sunken into a pit within the flagellum, as well as the thick, inflexible socket are thought to preclude the excitation of the receptor cells through external mechanical stimuli, therefore, supporting the above-mentioned hypothesis (mechanical hygrometer model) on stimulus perception (Altner and Loftus, 1985). In addition, some authors have indicated structural similarities between thermo-/hygro-sensitive np-ns sensilla regarding the abundance of cytoskeletal elements in the form of longitudinally oriented microtubules, together with the thick ds and the scolopale rods in mechanosensitive, amphinematic scolopidia (Yokohari, 1981). These similarities would also be in line with the mechanical hygrometer model. As an alternative model for humidity transduction the psychrometer model has been proposed, in which the degree of cooling during evaporation of water is used to measure the humidity of the air (Tichy and Kallina, 2013). According to this model it is expected that both, the moist cell’s and the dry cell’s discharge rates should increase with rising temperature. This specific

![Fig. 10. Dose-response curves of the different receptor cells. Neuronal responses of three individual cold (a) and warm cells (b) to different rates of temperature changes and of two individual dry (c) and moist cells (d) to different rates of changes in relative humidity. Rates of stimuli changes (x-axes) are given on a logarithmic scale. Equations describe the corresponding logarithmic regression lines.](image)

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Table 2
Overview of co tested electrophysiologically. For each sensillum, numbers of sensory cells and their respective primary sensory modality are given, based on their responses to temperature and humidity oscillations.
temperature dependence of the humidity response was indeed observed in thermo-/hygrosensitive antennal sensilla of the cockroach, *Periplaneta americana* (Tichy and Kallina, 2013), and would also be in line with the physiological responses of the hygroreceptors observed in our present study in *Mecopoda* sp. (cf. Fig. 8).

Stimulation of the proposed thermoreceptor in np-is sensilla through mechanical deformation of the dendrite has been excluded so far, due to its location beneath the peg, where the DOS was surrounded by lymph (Altner and Loftus, 1985). However, this is not the case for the co of *Mecopoda* sp. because the dendrites also fill the lumen of the peg. Therefore, a primary mechanosensitivity could be proposed for the thermoreceptors, at least in *Mecopoda* sp. The dendrites could be stimulated by thermomechanical deformations of the peg wall or other cuticular components. The insect cuticle, like most organic molecules, strongly absorbs electromagnetic radiation, especially in the mid-(3–8 μm) and long-wave infrared region (8–15 μm) (Hesse et al., 1995; Vondran et al., 1995), whereby the energy of the photons is converted into heat. Any heating inevitably causes thermal deformation. Since the penetration depth of infrared photons into cuticle is assumed to be in the range of 3–4 μm (Schmitz et al., 2016), not only the peg wall, but also the thick ds itself and the electron-dense structure bordering it, could function as infrared-absorbing structures and lead to an excitation of the thermoreceptor through thermally induced deformations.

A similar functional principle of thermosensation can be found in the so-called photomechanical, infrared receptors of pyrophilous *Melanophila* beetles (Schmitz et al., 1997) and true bugs of the genus *Aradus* (Schmitz et al., 2008), and has also been discussed for the infrared organs of *Merimina atrata* (Schneider and Schmitz, 2013, 2014), which are morphologically entirely different from the former (for a review, see Schmitz et al., 2016). In these receptors, infrared radiation is absorbed by the cuticular apparatus, which show distinctive morphological adaptations, and finally leads through thermomechanical deformations of the cuticular apparatus to excitation of a mechanoreceptor.

We found a thick ds that is continuous with the inner peg wall, which seems to be present in many thermo-/hygrosensitive np-is sensilla (Altner et al., 1981, 1983; Steinbrecht and Müller, 1976; Yokohari, 1983). One could speculate on its potential function as a heat-conducting structure that directs the heat absorbed by the peg towards the thermoreceptive site. Such a function has been proposed recently for the infrared receptors of pyrophilous *Acanthocnemus nigricans* beetles on the basis of finite-element simulations (Zhou et al., 2016). In these receptors, the so-called rod is believed to function as a thermal conductor and most probably represents a hypertrophied ds (Kreiss et al., 2005). However, direct evidence is lacking.

Whether the receptor cells and structural components that we identified in the co of *Mecopoda* sp. in the present study really function in the way described above is currently a matter of speculation and further investigation is required. Alternatively, the neurons could also be directly excited through the temperature-activated ion channels of the transient receptor potential (TRP) family (thermoTRPs) (Hamada et al., 2008; Saito and Tominaga, 2015).

4.2. Potential role of antennal sensilla coeloconica (co)

The responses of the thermosensitive co in *Mecopoda* sp. to convective heat showed that an increase in the resting activity of the cold and the warm cells by 1 imp/s can be elicited by a rate of temperature change of approximately −0.008 to −0.051 °C/s and +0.006 to +0.011 °C, respectively (Fig. 10). These sensitivity values are within the same order of magnitude as those reported for other thermoreceptive sensilla in the literature (e.g. *A. aegypti*: Davis and Sokolove, 1975; *A. variegatum*: Hess and Loftus, 1984; *Atta vollenweideri*: Ruchty et al., 2010; *Camponotus rusipes*: Nagel and Kleineidam, 2015; *Cupiennius salei*: Ehn and Tichy, 1996; *R. prolixus*: Zopf et al., 2014; *S. lucidulus*: Corbière-Tichané and Loftus, 1983).

However, with respect to the “hot-male” hypothesis, the responses of the receptor cells to thermal stimuli in the form of radiant heat are much more interesting, since heat transfer does not depend on convection (particle movement in air and, therefore, on wind direction). This would be a required property for a reliable, contactless localization of thermal stimuli from a given distance. Although both heat sources, convective as well as radiant heat, lead to a distinct warming of the stimulus-receiving structures, they are based on different physical mechanisms. This might be one reason for the differential sensitivity of thermosensitive sensilla to convective and radiant heat, which is probably based — at least to a certain degree — on differences in the morphology of their cuticular apparatuses (Zopf et al., 2014). In the co of the mosquito *A. aegypti*, the warm and cold cell are reported to respond with a high degree of sensitivity to changes in convective heat (see above), but not to radiant heat (with maximum radiation intensity of approximately 1 μm) (Davis and Sokolove, 1975).

For the thermoreceptors in the co of *Mecopoda* sp., we could determine general excitability not only by stimulation with convective but also with radiant heat (maximum radiation energy at a wavelength of 9 μm). Although we were not able to determine a sensitivity threshold for the thermoreceptors to infrared radiation, the responses depicted in Fig. 9 clearly show that a radiation intensity of up to 20 W/m² (measured at the sensillum site), leading to an increase in temperature of +0.05 °C, still elicits a considerable change in the neuronal activity of both thermoreceptors. The same neuronal response as shown in Fig. 9 could also be elicited by a *Mecopoda* sp. male located at a distance of about 3 cm, with a thorax heated up by singing. For this calculation, we used the Stefan—Boltzmann law according to the Equation (1), assuming that 1) the *Mecopoda* sp. male has a thoracic area of 0.12 × 10⁻³ m², 2) after singing the thoracic temperature is +7.6 °C above ambient temperature (Erregger et al., 2017), 3) the emissivity (ε) of the cuticle is 0.97 (Stabentheiner and Schmaranzer, 1987) and 4) the radiation intensity at the sensillum site of the female measures 20 W/m². If we assume, as an example, a sensitivity threshold of the thermoreceptors in *Mecopoda* sp. of 0.6 W/m², as has been determined for the warm cell in the tapered hair of *R. prolixus* (Zopf et al., 2014), the same calculation would even result in a detection distance of a hot male of 17 cm.

Although our data indicate that *Mecopoda* sp. females are capable of detecting a singing male from distances of at least several centimetres solely by assessing thermal cues, the existence of such thermoreceptors (which most probably all insects have) cannot be regarded as a sufficient proof for the “hot-male” hypothesis. Furthermore, the fact that we could not find any sex specific differences regarding the number and distribution of thermosensitive co in *Mecopoda* sp. may indicate a more general function of these sensilla as, for example, in the context of thermoregulation and maintenance of a stable water-balance. Whether and to what extent thermal stimuli are used by *Mecopoda* sp. during mate choice can only be determined by sophisticated behavioural experiments.

Author contributions

E.S.S. and H.R. developed the conception and design of research. C.J.K. and E.S.S. designed the setup of electrophysiological
experiments. E.S.S. and G.L. designed the electron microscopic analyses. E.S.S. performed morphological and electrophysiological analyses, analysed and interpreted the data, wrote the main manuscript and prepared figures and tables. All authors discussed the results and reviewed the manuscript.

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