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RESEARCH ARTICLE

Polarization sensitivity in Collembola: an experimental study of polarotaxis in the water-surface-inhabiting springtail *Podura aquatica*

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ABSTRACT

The ventral eye of the water-surface-inhabiting springtail *Podura aquatica* has six ommatidia with horizontal and vertical microvilli and perceives light from the ventral, frontal and frontodorsal regions, whereas the dorsal eye possesses two upward-looking ommatidia with vertical microvilli. The ventral eye may detect water by its polarization sensitivity, even if the insect is resting with its head slightly tipped down on a raised surface. The polarization sensitivity and polarotaxis in springtails (Collembola) have not been investigated. Therefore, we performed behavioural choice experiments to study them in *P. aquatica*. We found that the strength of phototaxis in *P. aquatica* depends on the polarization characteristics of stimulating light. Horizontally and vertically polarized light were the most and least attractive, respectively, while unpolarized stimulus elicited moderate attraction. We show that horizontally polarized light attracts more springtails than unpolarized, even if the polarized stimulus was 10 times dimmer. Thus, besides phototaxis, *P. aquatica* also performs polarotaxis with the ability to measure or at least estimate the degree of polarization. Our results indicate that the threshold d^* of polarization sensitivity in *P. aquatica* is between 10.1 and 25.5%.

KEY WORDS: Collembola, Springtail, *Podura aquatica*, Polarization sensitivity, Polarotaxis, Water detection, Visual ecology

INTRODUCTION

Springtails (Collembola) are abundant in all continents, even in the extreme conditions of Antarctica. The majority of the almost 7000 Collembola species form an important part of terrestrial ecosystems. They live in the soil, feed on decaying plant matter and soil fungi (Rusek, 1998). However, some species, like *Podura aquatica* Linnaeus 1758, inhabit water surfaces (Shaller, 1972; Kriska, 2013). It has been shown that *P. aquatica* springtails strongly depend on water as they can be easily dehydrated through their thin cuticle by transpiration and damage to the cuticle increases the transpiration rate. Restoring the speed of water loss to the normal level is achieved by regular moulting (Noble-Nesbitt, 1963a,b).

Generally, the cuticle of *P. aquatica* is unwettable and the water surface acts as a membrane on which springtails can walk. Springtails submerged by water waves are surrounded by a thin silvery air layer, the buoyant force of which lifts them back to the water surface. On their first abdominal segment they have a hydrophilic ventral tubular appendage called the collophore, the main functions of which are excretion, water intake and adhesion to the water surface (Noble-Nesbitt, 1963c; Hopkin, 1997). Collembola, especially water-inhabiting species like *P. aquatica*, also possess a forked unique locomotory organ, called the furcula, attached to the fourth abdominal segment. The furcula is generally folded under the body, but when released, it snaps backwards and springs the animal upward providing a quick escape from predators (Hopkin, 1997; Kriska, 2013).

Aquatic insects detect water by means of the horizontal polarization of water-reflected light and are guided to their water habitats by polarotaxis (Schwind, 1983, 1984, 1989, 1991, 1995, 1999; Wildermuth, 1998; Horváth and Varjú, 2004; Csabai et al., 2006; Manor et al., 2009; Horváth et al., 2008; Egri et al., 2012; Horváth and Csabai, 2014). Until now, the polarization sensitivity and polarotaxis of Collembola have not been investigated.

Previous studies have demonstrated that the photoreceptors in several springtail species also possess microvillar arrangements that may enable them to perceive light polarization (Paulus, 1972; Meyer-Rochow et al., 2005). The phototactic behaviour of various Collembola species has been studied, and the results showed negative phototaxis except in species living on water surfaces or plants (Shaller, 1972; Salmon and Ponge, 1998; Dromph, 2003; Fox et al., 2007), such as *P. aquatica*. The ecological reason for negative phototaxis in the majority of springtails is that they live in the soil and light indicates an inappropriate habitat that should be avoided. In addition to phototaxis, geotaxis (Boiteau and MacKinley, 2014) and shape perception (Shaller, 1972) have also been demonstrated in Collembola.

The number of ommatidia in the eyes of springtails varies within species from a maximum of eight to their total absence. *Podura aquatica* has eight ommatidia in a 'double eye' partitioned into a dorsal and ventral eye region, and the orientation of each ommatidium is also known (Paulus, 1970). The ventral and dorsal eye regions are composed of six and two ommatidia, respectively. The ventral eye region is equipped with strictly horizontal and vertical (orthogonal) microvilli and perceives the light from the (i) ventral, (ii) frontal and (iii) frontodorsal regions, whereas the two upward-looking dorsal ommatidia possess only vertical microvilli (Fig. 1A). Owing to the wide (up to 80 deg) opening angles of the collembolan ommatidia (Shaller, 1972), the field of view of the ventral eye region is presumably not limited to the lower hemisphere relative to the head; however, the exact opening angles of the ommatidia in *P. aquatica* has not been studied. Hence, it is presumable that the ventral eye region may also serve to detect water by its polarization sensitivity, even if the animal is resting with its head slightly tipped down on a raised surface (Fig. 1B).

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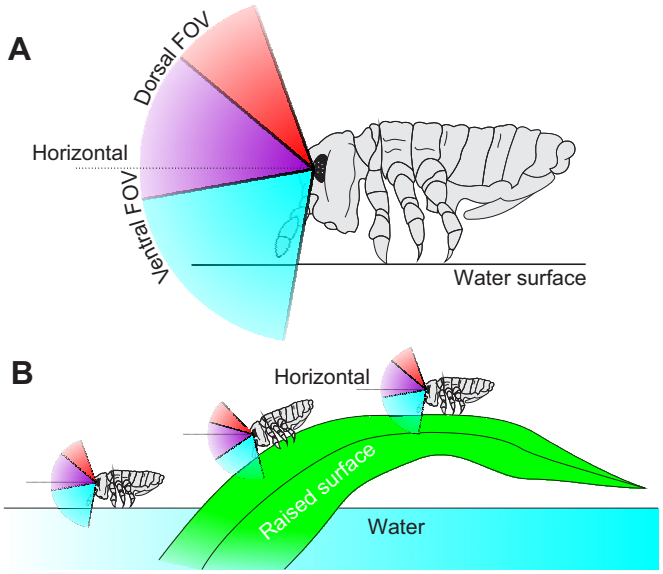

Fig. 1. The field of view of the 'double eye' in *Podura aquatica*. (A) The slightly overlapping (violet) red and blue sectors represent the estimated field of view (FOV) of the dorsal and ventral eye regions, respectively.
(B) Demonstration of the role of the ventral eye region in water detection, even if the head is tipped down. The green leaf represents an arbitrary raised surface (e.g. soil, vegetation, gravel).

Even though orthogonally aligned microvilli are present in the ventral eye region of the water springtail *P. aquatica*, it does not follow that this species possesses polarotaxis, although this is a reasonable hypothesis because of its strong dependence on water. Therefore, we performed behavioural choice experiments to study the polarization sensitivity and polarotaxis in this collembolan species.

MATERIALS AND METHODS

Springtails

Podura aquatica adults (males and females) were collected from the surface of ponds and creeks in the vicinity of Budapest, between March and June 2015. The springtails were kept in the laboratory at 10°C under 12 h:12 h dark:light conditions in jars containing original pond-water and aquatic plant leaves.

Choice box

The primary equipment of our experiments was a choice box possessing two windows for light stimuli with variable polarization characteristics (Fig. 2). The arena was composed of a small aquarium (30×20×10 cm), the inner and outer surface of which was covered with matte white paper except for two square (5.6×5.6 cm) areas on the two ends of the aquarium making up windows for the light stimuli (Fig. 2A,B). The matte white paper ensured the minimization of specular reflections and unwanted polarization signals. The choice box had a removable cover with a circular hole through which the interior of the arena could be recorded by a digital camera (Fig. 2A). The inner surface of the cover was also matte white; thus, the tested springtails moving at the arena bottom saw a homogeneous matte white environment except for the two stimulus windows and the objective lens of the camera at the centre of the top element (Fig. 2B). On the bottom of the choice box was an exchangeable matte white sheet of paper with two printed black lines dividing the box into three equal partitions and a printed black circle at the centre of the paper representing the release location of springtails.

Depolarizer array

The polarization characteristics of each light stimulus were variable discretely by means of a linearly polarizing sheet (XP42-18, ITOS, Mainz, Germany) housed in a rigid cardboard frame and a series of 15 slightly depolarizing sand-blasted glass panes between two

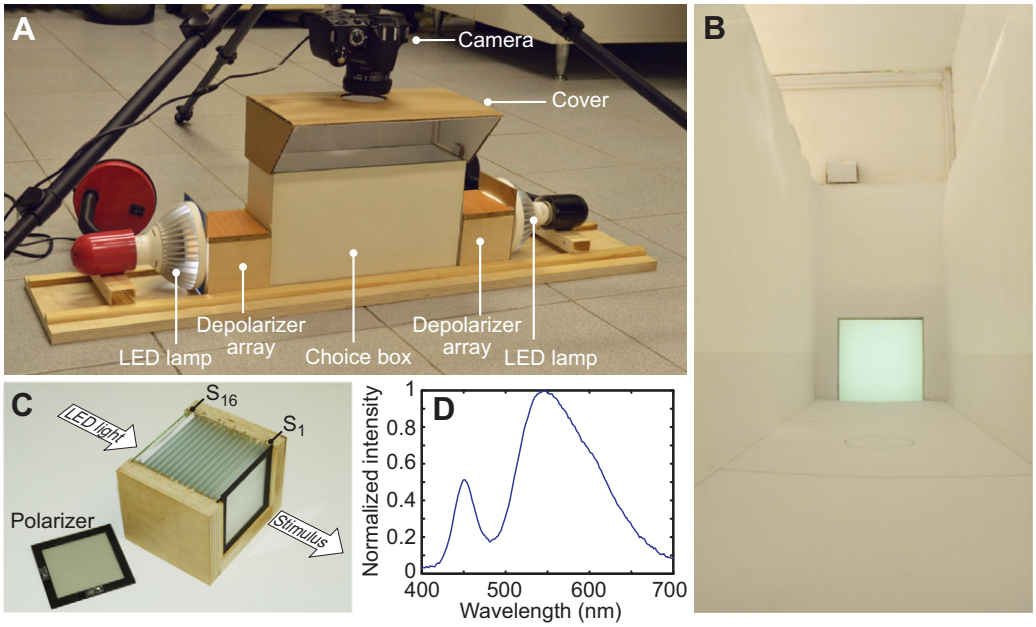

Fig. 2. Overview of the experimental setup used for the choice experiments. (A) Photograph of the setup. (B) Perspective from the point of view of a springtail from one end of the choice box. The laboratory lights were turned off during the experiments. (C) A linear polarizer is shown along with the rigid frame and the structure of the depolarizer array composed of two ordinary and 15 sand-blasted glass panes in position in the U-shaped profile. In slot S_{15}, two layers of matte white office paper are inserted. (D) Emission spectrum of the light stimulus entering the choice box.

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 2567-2576 doi:10.1242/jeb.139295

ordinary, colourless, transparent, non-polarizing and non-depolarizing glass layers fixed in a wooden U-shaped profile (Fig. 2C). Therefore, 3 mm wide gaps between the neighbouring glass layers formed 16 slots, where the frame with the polarizer could be inserted. The white unpolarized light emitted by a Ledion LB-P38-153100 cool LED lamp (640 lumens) entered the U-shaped profile and penetrated through all of the glass layers and the polarizer. The transmission axis of the polarizer in the frame and the number of slots the frame was slipped into (S_i, i=1…16) determined the angle α and degree d (%) of polarization of the stimulus: the closer the polarizer was to the LED lamp, the lower the d of light stimulus was, because the light must have passed more depolarizing glass layers after leaving the polarizer. Two layers of matte white paper were also inserted into slot S_{15} (second slot from the LED lamp) to ensure the total depolarization of light (Fig. 2C). Consequently, inserting the polarizer into slot S_1 (furthest from the LED lamp) or S_{16} (closest to the LED lamp) created 100% linearly polarized or practically unpolarized light stimulus, respectively. Finally, a wooden lid covered the depolarizer array at the top. We prepared 10 frames holding a polarizer sheet, each with differently orientated transmission axis, thus the angle of polarization α could be varied with a 10 deg step between the horizontal (α=0 deg) and vertical (α=90 deg) by inserting the proper polarizer-holding frame into the desired slot of the depolarizer array.

The degree of polarization d of the stimulus was measured as a function of the polarizer position in the red (650±50 nm), green (550±50 nm) and blue (450±50 nm) parts of the spectrum with a Nikon D3200 digital camera equipped with a calibrated polarizer (W-Tianya Slim MC CPL). Shooting images with three polarizer angles in RAW format [the linear voltage response of the CMOS pixels as a function of light intensity as recorded in the RAW image was verified by Estrato Research & Development (www.estrato.hu)] enables the experimenter to calculate the degree and angle of polarization pixel by pixel (Horváth and Varjú, 1997, 2004). At each slot setting, the d values obtained at the pixels corresponding to the stimulus window were averaged and the results of the three spectral bands were also averaged. Fig. 3 shows the d and α patterns of an unpolarized (Fig. 3A–C), 100% horizontally polarized (Fig. 3D–F) and 100% vertically polarized stimulus (Fig. 3G–I) with the choice box interior in the green (550 nm) spectral range.

Independent of the polarizer position, the spectral characteristics of the light stimuli were the same, since the same materials of the same number occupied the optical path. The emission spectrum of the light stimuli, which was measured with an Ocean Optics STS-VIS spectrometer in the visible spectral range, had a major and a minor peak at 550 and 450 nm, respectively (Fig. 2D). However, the intensity of the light stimulus had a slight dependence on the polarizer position, because the frame of the polarizer did not block the whole cross-section of the depolarizer array near the bottom of the lid. We measured this dependence for horizontally and vertically polarized stimuli by taking photographs from the other stimulus window in RAW format with the same camera settings, and finally summing all pixel values for each image. Then, we normalized the total intensity values with the 100% polarized case which was the maximal value. As Fig. 4A shows, we obtained a monotonic increase in intensity from the unpolarized to the 100% polarized case and the ratio of the two extremes was I_{unpol}/I_{pol}=0.84. The difference between the vertically and horizontally polarized calibration curves was negligible.

To test how the matte white coating affects light reflection as a function of polarization, we also measured and compared the total wall-reflected intensities with the direct stimulus intensities in case of 100% horizontally and 100% vertically polarized stimulus as a function of the degree of polarization (polarizer position). Fig. 4C

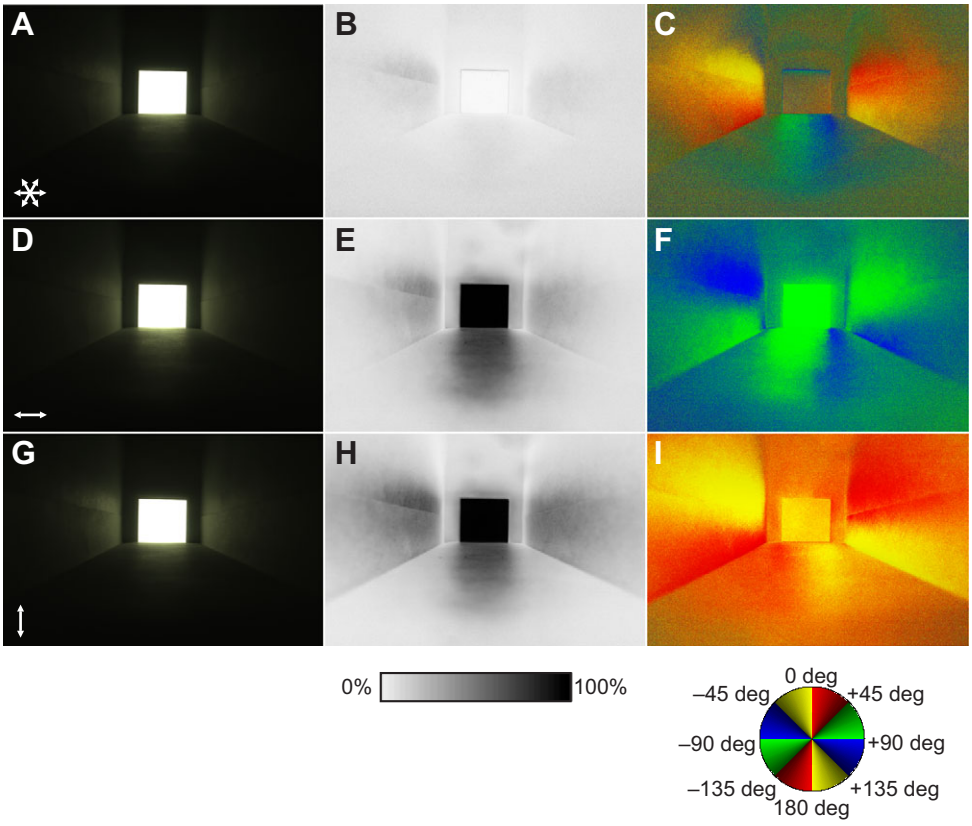

Fig. 3. Imaging polarimetry of the interior of the choice box. Unpolarized (A–C), 100% horizontally polarized (D–F), and 100% vertically polarized (G–I) stimulus in the green (550 nm) spectral range. (A,D,G) Original RGB photographs. (B,E,H) Patterns of degree of linear polarization. (C,F,I) Patterns of angle of polarization measured clockwise from the vertical.

Journal of Experimental Biology (2016) 219, 2567-2576 doi:10.1242/jeb.139295

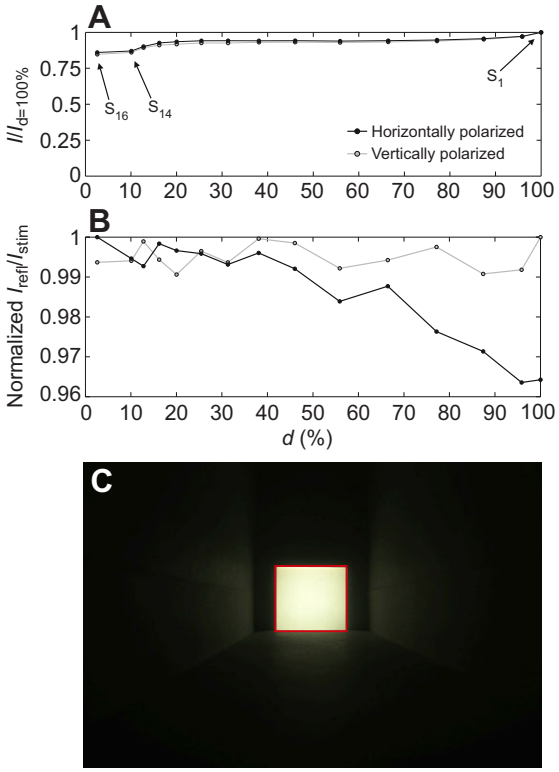

Fig. 4. Intensity of the stimulus and reflections on the walls as a function of the degree of polarization *d* (%) for horizontally (black lines) and vertically (grey lines) polarized light. (A) Normalized intensity of light stimulus and reflections together. (B) Normalized ratio of the intensity of reflections and direct stimulus (I_{refl}/I_{stim}). (C) Example of a RAW image of the choice box interior with an unpolarized stimulus. The sum of the pixel values outside and inside the red rectangle was used to calculate I_{refl}/I_{stim}.

shows a RAW image with the stimulus window and the interior of the choice box. The ratio of the summed pixel values of the reflections (outside the red rectangle) and the direct stimulus (inside the red rectangle) was calculated for each image. Dividing with the maximum value resulted in the normalized I_{refl}/I_{stim} ratio as a function of the degree of polarization (Fig. 4B). It is clear that the intensity ratio of the reflections and the direct stimulus was significantly less than 4% in the case of the majority of the polarizer positions. In other words, the Weber contrast between the reflection-related disturbances and the direct stimulus was significantly low.

Test trials

The tests with *P. aquatica* were performed in choice trials. At first, to minimize the influence of odours, a new matte white paper sheet with the black partitioning lines was placed onto the bottom of the choice box, and an opaque plastic releaser tube (diameter=28 mm, height=14 cm) was stood in the centre of the box. Around 100-250 *P. aquatica* specimens were placed in the releaser, the cover of the choice box was set up, the desired light stimuli were applied (Fig. 2C,D) and the laboratory was darkened. After 30 s, the releaser was removed and 10 photographs (6016×4000 pixel resolution, JPEG format) were taken over 81 s. Then, the cover was detached and the springtails were collected from the choice box. In order to eliminate artefacts arising from the incidental slight differences in the LED light sources and the two sides of the arena, each trial was repeated with reversed stimulus arrangement. Thus, we measured Collembola reactions to different stimulus pairs in even numbers of trials and equal numbers of trials were carried out for each stimulus configuration. Furthermore, to avoid pseudo-replication, new specimens were always introduced in each trial. In this way, a total of 25,407 *P. aquatica* specimens were tested in 5 experiments covering 164 trials (Table 1). An additional 300 springtails were also tested individually in experiment 6, as described later. The relative humidity was measured with a HIH-4000 Series humidity sensor in the laboratory and varied between 45 and 50% during the experiments.

Table 1. Number of *P. aquatica* springtails tested and number of trials in the six laboratory choice experiments

Experiment	Number of springtails	Number of trials
1	1727	10
2	3342	24
3	2470	18
4	10,334	72
5	7534	40
Sum of experiments 1–5	25,407	164
6	300	300

Evaluation and statistics

In the 10 photographs taken during every trial (Fig. 5A–C), the position of each springtail (being the only non-static objects in the arena) was determined by a custom-developed software written in GNU Octave v.4.0 (Fig. 5; for details of the algorithm and the software, please contact the corresponding author). For each trial, as the first step, a static background image of the choice box was obtained by calculating the median of the 10 images (Fig. 5E). Subtracting the inverse of the background image from the inverse of a given photograph resulted in a new image containing only the springtails as bright patches on a black background (Fig. 5F). This image was thresholded using the method of Otsu (1979), and the number and centroid position of the patches were determined (Fig. 5G). The two black lines on the underlying white paper perpendicular to the longer edge of the choice box were also recognized by the software, thus it could be determined automatically if a given springtail was located in the left, middle or right third of the choice box (Fig. 5I). To minimize errors, the detection of springtails was checked manually in the case of all photographs, and the threshold level was adjusted if it was necessary.

In the first photograph (t=0 s), the springtails were crowded at their starting position (black circle). Later, they dispersed and shortly, several specimens approached the wall of the choice box, and a few got under the replaceable paper sheet. Since the automatic detection underestimated the number of springtails when they were initially crowded at a relatively small area, the total number of specimens was determined correctly later, when they dispersed, but before they had time to get under the paper. Thus, for each trial, the maximal number of detections from the 10 images was considered as the number of springtails participating in the given trial. Fig. 5H shows the mean number of detected springtails as a function of the image number for all 164 trials. The maximal value occurred at file number 6 (t=45 s), thus the chosen 81 s duration for the trials was justified.

In order to quantify the reaction strength of the several hundred *P. aquatica* at a given stimulus setting, we calculated the mean position shift of springtails toward one of the sides (e.g. polarized stimulus) relative to the centre of the choice box for the last photograph (t=81 s) corresponding to the given stimulus pair. For example, in experiment 4, we tested the preference of springtails for

Journal of Experimental Biology (2016) 219, 2567-2576 doi:10.1242/jeb.139295

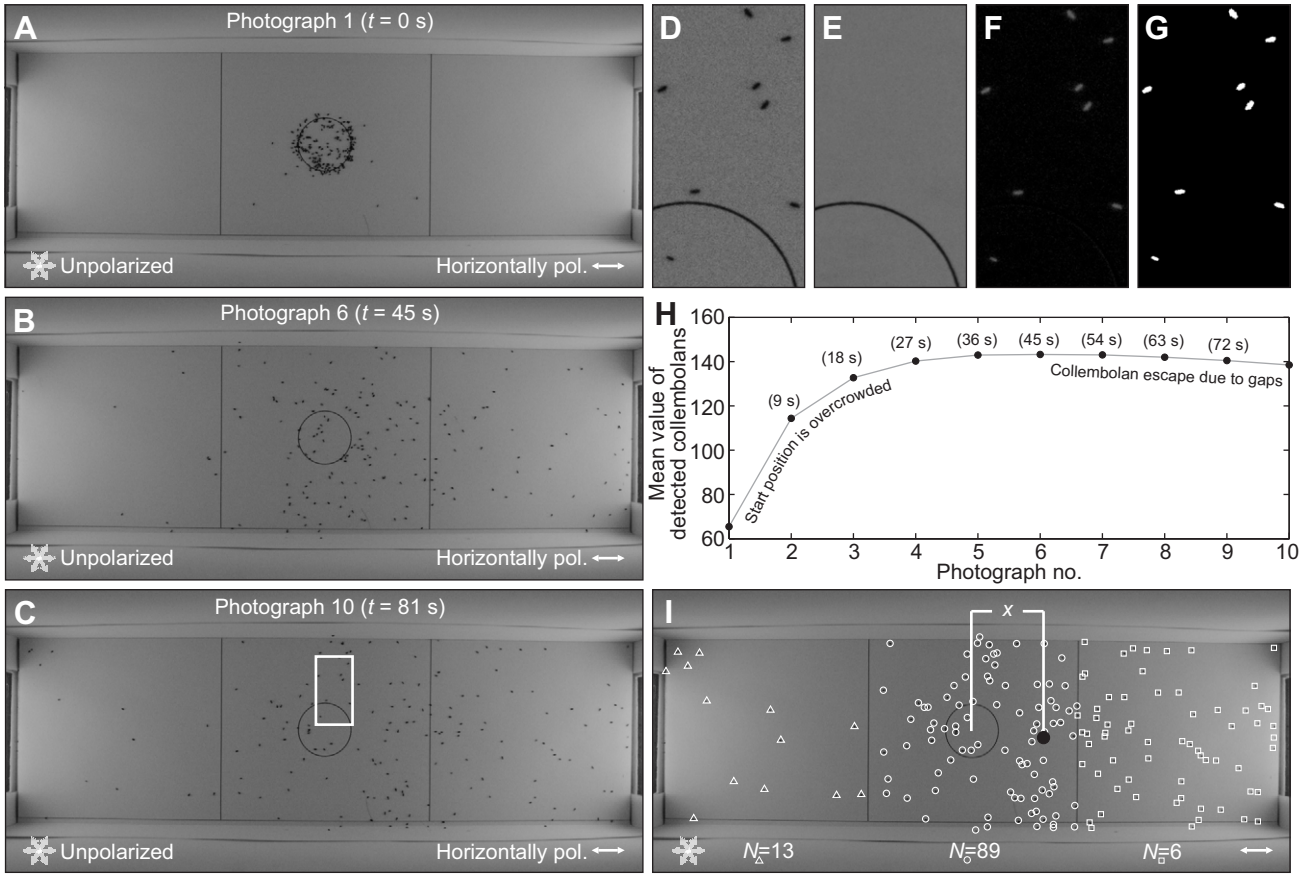

Fig. 5. Evaluation process in the case of a trial where the left stimulus was unpolarized and the right was 100% polarized. (A–C) 1st (*t*=0 s), 6th (*t*=45 s) and 10th (*t*=81 s) photograph of the trial. (D) Original 10th photograph (magnified image from C). (E) Median of the 10 photographs taken during the trial. (F) The inverse of E subtracted from the inverse of D. (G) Thresholded image of F. (H) Number of detected springtails as a function of photograph number taken during the 81 s test averaged for all 164 trials. The elapsed seconds are also shown in brackets. (I) Results of the detection process. Triangles, circles and squares show the detected springtails in the left, middle and right third of the choice box, respectively. The black circle shows the centroid of all detected springtails and *x* is the shift of the centroid of springtail positions in pixels.

polarized light against unpolarized one in four trials in each stimulus configuration. Thus, for a given stimulus pair we calculated the centroid of springtail positions toward the polarized stimulus, including all four photographs taken at *t*=81 s (in case of swapped stimulus settings, the horizontal coordinates were multiplied by −1). We defined the relative centroid shift Δx as:

$$\Delta x = x/L, \quad (1)$$

where x is the horizontal coordinate of the centroid of springtail positions, and L is the length of the choice box, both measured in pixels. The other quantification method we used for determining the significance of reactions in a given stimulus setting was to compare the number of springtails in the two terminal thirds of the arena at the end of the trial (last photograph, *t*=81 s) with a χ^2 test. The specimens in the middle third were treated as inactive and were ignored, even though they were moving. Presuming a linear relationship between light intensity and the strength of phototaxis, for the χ^2 tests, for experiments 4, 5 and 6, we modified the expected number of responses linearly proportional to the intensities of the two stimuli (Fig. 4A) in order to compensate for the slight intensity differences. For example, in experiment 6, when 100 springtails were tested and the stimuli were unpolarized and 100% horizontally polarized, the expected number of responses were modified to 45.652 and 54.348 based on the I_{unpol}/I_{pol}=0.84 intensity ratio (Fig. 4A).

Experiment 1: control

In order to test the homogeneity of the choice box, we performed control trials in which both optical stimuli were unpolarized with equal intensity. On both sides of the choice box the polarizer was inserted into slot S_{16} of the depolarizer array to produce unpolarized stimulus (with degree of polarization d≈0%).

Experiment 2: phototaxis

In this experiment, we tested the phototactic reactions of *P. aquatica* in three cases: at one side of the choice box, the LED light source was turned off (dim stimulus), and the other stimulus was (i) 100% horizontally polarized light, (ii) 100% vertically polarized light, or (iii) unpolarized light with operating LED light (polarizer inserted into slot S_1).

Experiment 3: polarotaxis versus phototaxis

Here, we tested the preference of *P. aquatica* to 100% horizontally polarized light against unpolarized light with dimmer light intensities on the polarized side of the arena. The intensity ratio I_{pol}/I_{unpol} of the polarized and unpolarized stimulus varied between 0.063 and 1.140. The intensity of the polarized stimulus was changed by inserting an additional frame containing a polarizer

Journal of Experimental Biology (2016) 219, 2567-2576 doi:10.1242/jeb.139295

sheet with different, oblique transmission axes into slot S_2, next to the horizontal polarizer placed in slot S_1. According to Malus law, the transmitted intensity of 100% polarized light through a linear polarizer is proportional to $\cos^2\beta$, where β is the angle between the direction of polarization of incoming light and the transmission axis of the polarizer. In this way, the transmission axis of the polarizer in slot S_2 determined the intensity of light stimulus exiting the horizontal polarizer in slot S_1. The exact intensities were measured with the same digital camera by extracting the pixel information of the stimulus window from RAW images. The outcome of this experiment revealed whether *P. aquatica* possesses polarization vision, or if only the strength of the horizontally polarized component of the stimulus influences its reaction.

Experiment 4: varying degree of polarization *d*

In this experiment, we tested the preference of *P. aquatica* to horizontally and vertically polarized light against unpolarized light as a function of the degree of polarization *d*. Different *d* values were produced by using slots S_1, S_2, S_3, S_4, S_5, S_6, S_8, S_{10}, S_{14} and S_{16} of the depolarizer arrays. The resulting *d* values (averaged over the visual spectral range) were 100.0, 95.9, 87.4, 77.2, 66.5, 55.9, 38.1, 25.5, 10.1 and 2.6%, respectively. These values are the averages of pixel-by-pixel measurements in three spectral bands (R, G, B) as described above. The standard deviation was less than 2.5% in all cases. The ratio of the intensities of the unpolarized and polarized stimuli is shown in Fig. 4A as a function of *d*.

Experiment 5: varying the angle of polarization

In this experiment, we tested the reaction of *P. aquatica* to a varying angle of polarization of 100% polarized light against an unpolarized stimulus. The ratio of the intensities of the unpolarized and polarized stimuli was I_{unpol}/I_{pol}=0.84 (Fig. 4A). α was changed between the horizontal and vertical in 10 deg steps.

Experiment 6: tests with individual springtails

As numerous springtails were involved simultaneously in each trial, the question arises whether the behaviour of a given springtail might have been affected by others. The ideal method would be to test each springtail separately, independent of the others. However, this technique would be impractical because of the thousands of specimens. To show that the reactions were not appreciably affected by the presence of other specimens in the choice box, we performed experiment 6, in which we introduced the springtails one by one. We tested three situations each with 100 springtails: (i) 100% horizontally polarized versus unpolarized light, (ii) 100% vertically polarized versus unpolarized light, and (iii) unpolarized versus unpolarized stimulus as a control experiment. The ratio of the intensities of the unpolarized and polarized stimuli (I_{unpol}/I_{pol}) was 0.84, and was equal to 1 in the third case. After release, at t=81 s, the position (left, right or middle partition) of the single springtail was registered visually through the circular hole on the cover. The stimulus arrangement was swapped after every fifth test.

RESULTS

The results of our experiments provided detailed information about the polarization sensitivity as well as polarotactic and phototactic behaviour of *P. aquatica* in the visible spectral range. Table 1 shows the numbers of trials and tested *P. aquatica* in our six experiments. Table 2 contains the measured relative centroid shift Δx of springtail positions in experiments 1 and 2 with the statistical significance of reactions.

In experiment 1, we tested the homogeneity of the choice box in control trials. It is clear from Table 2 that the value of Δx was practically zero, and left–right reactions of springtails showed no significant difference (χ^2=0.54, d.f.=1, P=0.4624). Hence, the attractiveness of both identical unpolarized stimuli was the same to Collembola.

The results of experiment 2 show unambiguous positive phototaxis in *P. aquatica*. However, the reaction strength depended on the polarization characteristics of the light stimulus. According to Table 2, springtails preferred the bright side of the choice box against the dim side. The relative centroid shift Δx toward the polarized stimulus was 0.0847, 0.0576 and 0.0186 when the light stimulus was 100% horizontally polarized, unpolarized and 100% vertically polarized, respectively. According to the χ^2 tests, the reactions were significant, except for the last one (Table 2).

Fig. 6 shows the reactions of springtails as a function of the intensity ratio of the polarized and unpolarized stimulus in experiment 3, where the phototaxis was compared with polarotaxis. The exact number of choices at the terminal thirds and the relative centroid shift Δx toward the 100% horizontally polarized stimulus are shown in Fig. 6A,B, respectively. The springtails were most attracted to the polarized stimulus when the intensity ratio of the polarized and unpolarized stimulus was maximal (I_{pol}/I_{unpol}=1.14). As the intensity of the polarized stimulus decreased, its attractiveness dropped also and became zero when the polarized stimulus was more than 10 times dimmer than the unpolarized one. At intensity ratio I_{pol}/I_{unpol}=0.063, the phototaxis

Table 2. Number of individual springtails observed in the terminal thirds of the choice box in experiments 1, 2 and 6

Experiment	Stimulus	Choices		Δx	χ^2	d.f.	P
1	Unpolarized versus unpolarized	N_{left}=259	N_{right}=276	0.0009 (toward right stimulus)	0.54	1	0.4624
2	100% horizontally polarized versus dim	N_{pol}=311	N_{dim}=118	0.0847 (toward polarized light)	86.83	1	<0.0001
	Unpolarized versus dim unpolarized	N_{unpol}=522	N_{dim}=283	0.0576 (toward brighter light)	70.96	1	<0.0001
	100% vertically polarized versus dim	N_{pol}=188	N_{dim}=165	0.0186 (toward polarized light)	1.50	1	0.2209
6	100% horizontally polarized versus unpolarized	N_{pol}=47	N_{unpol}=7	–	37.23*	1	<0.0001
	100% vertically polarized versus unpolarized	N_{pol}=9	N_{unpol}=47	–	33.02*	1	<0.0001
	Unpolarized versus unpolarized	N_{left}=28	N_{right}=23	–	0.49	1	0.4838

Asterisks indicate if the expected values were modified based on the slight intensity differences in the stimuli. The relative centroid shifts Δx of springtail positions for experiments 1 and 2 are also given in the table. Results were classed as significant when P<0.05.

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 2567-2576 doi:10.1242/jeb.139295

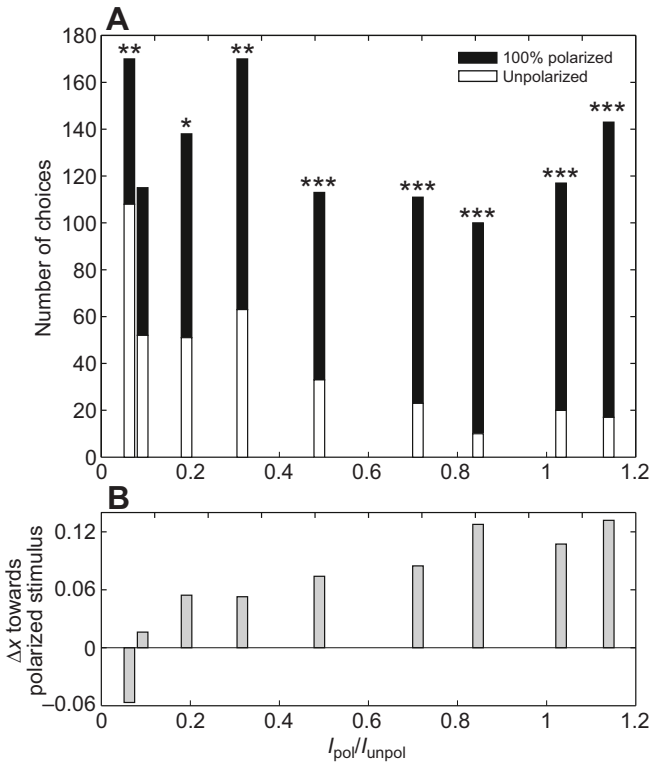

Fig. 6. Responses of springtails to the polarized and unpolarized stimulus (experiment 3) as a function of the intensity ratio I_{pol}/I_{unpol}. (A) Number of choices at the terminal thirds of the choice box corresponding to the 100% polarized (black bars) and unpolarized (white bars) stimuli. *P<0.05, **P<0.001, ***P<0.0001, χ^2 test. (B) Relative centroid shift Δx of springtail positions toward the 100% horizontally polarized stimulus.

overwhelmed the polarotaxis and the springtails preferred the unpolarized stimulus.

In experiment 4, we studied the responses of springtails to horizontally and vertically polarized light as a function of the degree of polarization against an unpolarized stimulus. Fig. 7A shows the number of choices at the terminal thirds of the choice box corresponding to the polarized and unpolarized stimuli, whereas Fig. 7B displays the relative centroid shift Δx of springtail positions as a function of d. The black and grey bars correspond to the horizontally and vertically polarized stimulus (Fig. 7A,B) and the white ones to the unpolarized stimulus (Fig. 7A). In general, *P. aquatica* preferred the horizontally polarized light against the unpolarized one, while in the presence of vertically polarized and unpolarized stimuli they preferred the unpolarized light. The reaction strength of springtails increased with increasing d.

The reactions of springtails, when the angle of polarization preference was tested against the unpolarized stimulus in experiment 5, are shown in Fig. 8. Fig. 8A displays the number of choices at the terminal thirds corresponding to the 100% polarized and unpolarized stimuli, and Fig. 8B shows the relative centroid shift of springtail positions toward the polarized stimulus as a function of α of the 100% polarized stimulus. Springtails were most attracted to the horizontally polarized light (α=0 deg) and moved away from the vertically polarized stimulus (α=90 deg). In the case of intermediate α values, a transition occurred around α=50 deg, where the distribution of springtails showed no preference for any stimulus.

Table 2 shows the reactions of individual *P. aquatica* springtails tested in experiment 6. Springtails preferred the 100% horizontally polarized light against the unpolarized one (N_{pol}=47, N_{unpol}=7, $N_{inactive}$=46). At the same time, they were attracted to the unpolarized stimulus when the other was 100% vertically polarized (N_{pol}=9, N_{unpol}=47, $N_{inactive}$=44). In both cases, the differences were highly significant. There was no significant difference when both stimuli were unpolarized (N_{left}=28, N_{right}=23, $N_{inactive}$=49).

DISCUSSION

Before drawing conclusions from our results, it is important to ensure of the symmetry of the choice box. The suitability of our choice box was verified by the outcome of experiment 1, which showed no significant spatial bias in the springtail distribution between two optically equivalent unpolarized stimuli (first row of Table 2). In addition to verifying the positive phototactic behaviour of *P. aquatica* springtails (Shaller, 1972), in experiment 2, we showed that the strength of their attraction to light depends on the polarization characteristics. According to Table 2, the attraction

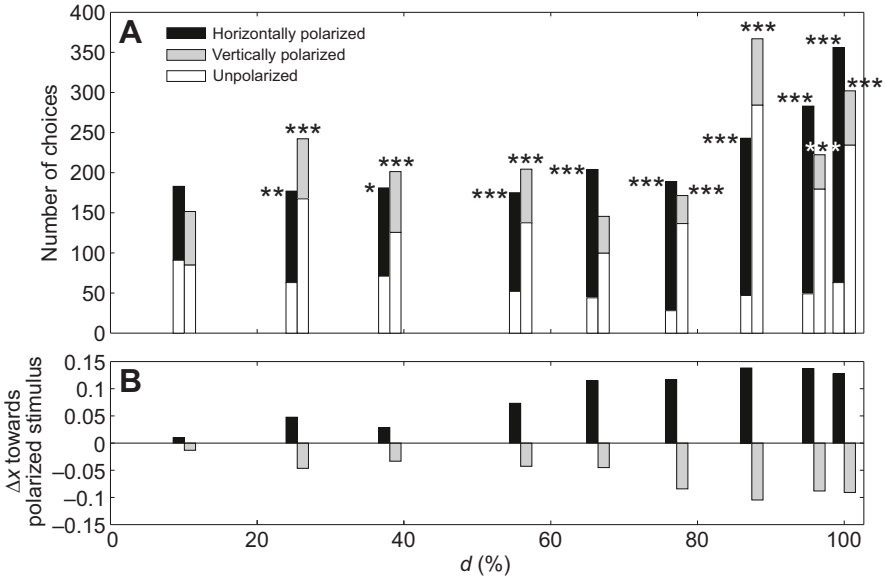

Fig. 7. Responses of springtails to horizontally and vertically polarized light in experiment 4 as a function of the degree of polarization *d* against unpolarized stimulus. (A) Number of choices at the terminal thirds of the choice box corresponding to the polarized (black bars, horizontal; grey bars, vertical) and unpolarized (white bars) stimuli. *P<0.05, **P<0.001, ***P<0.0001, in χ^2 tests performed with the modified expected values based on the slight intensity differences between the stimuli. (B) Relative centroid shift Δx of springtail positions toward the polarized stimulus.

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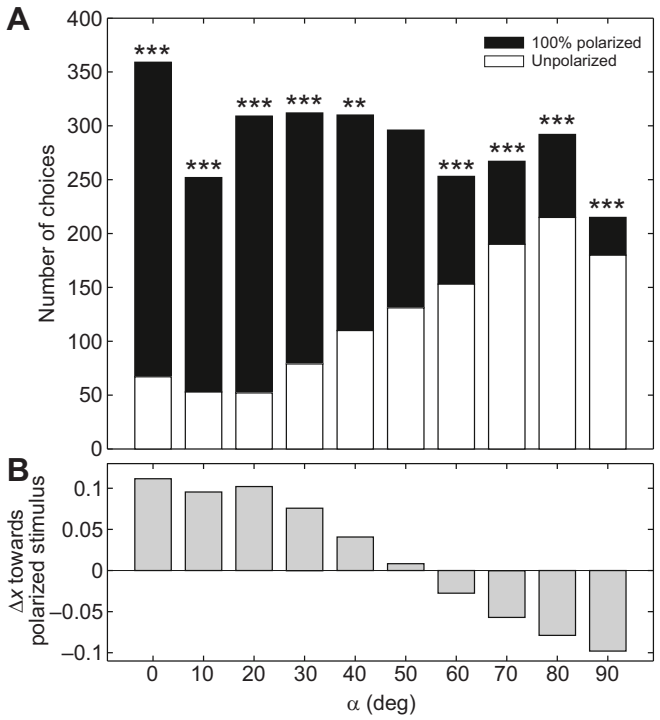

Fig. 8. Responses of springtails as a function of the angle of polarization from the horizontal (experiment 5). (A) Number of choices at the terminal thirds corresponding to the 100% polarized and unpolarized stimuli. $^{**}P<0.001$, $^{***}P<0.0001$, in χ^2 tests performed with the modified expected values based on the slight intensity differences between the stimuli. (B) Relative centroid shift Δx of springtail positions toward the 100% polarized stimulus.

was strongest and weakest when the bright stimulus was 100% horizontally and vertically polarized, respectively. The unpolarized stimulus elicited an intermediate, moderate attraction from springtails. Although the intensity ratio of the unpolarized and any kind of 100% polarized stimulus was $I_{unpol}/I_{pol}=0.84$, the comparison of the attraction to 100% horizontally and 100% vertically polarized light raises the reasonable suspicion that phototaxis and polarotaxis coexist in *P. aquatica*.

If only the horizontally polarized component of the light stimulus had played a role in the attraction of springtails, their distribution would have been symmetrical in the case of an intensity ratio $I_{pol}/I_{unpol}=1/2$ in experiment 3, because the horizontally polarized component of an unpolarized stimulus has half the intensity of the unpolarized stimulus itself. As shown in Fig. 6, Δx of springtail positions toward horizontally polarized light was positive, even if the polarized stimulus was 10 times dimmer than the unpolarized one. For each tested intensity ratio, the significances of the χ^2 tests are shown by asterisks in Fig. 6A. This fact obviously confirms the assumption, that in addition to phototaxis, polarotaxis is also present in *P. aquatica*, since they have the ability to measure or at least estimate *d* of the stimulating light. Similar coexistence of phototaxis and polarotaxis has been shown in numerous aquatic beetles. Furthermore, a synergistic interaction between both taxa has also been demonstrated (Boda et al., 2014).

Experiments 4 and 5 revealed more details about the nature of polarotaxis of *P. aquatica*. For polarotactic aquatic insects, the degree of polarization of water-reflected light is also a crucial parameter. In experiment 4 the springtails did not express any significant reaction if the polarizer was inserted into slot S_{14} (Fig. 7). From this, we conclude that the threshold of polarization sensitivity (d^*) in *P. aquatica* is between 25.5% (slot S_{10}) and 10.1% (slot S_{14}). The threshold of polarization sensitivity of the dorsal rim area in terrestrial field crickets (Labhart, 1996) and honey bees (von Frisch, 1967; Rossel and Wehner, 1984) is $d^*\approx5\%$ and $d^*\approx11\%$, respectively. In behavioural field tests, Kriska et al. (2009) measured d^* in polarotactic dragonflies ($d^*\approx0$–24%), mayflies ($d^*\approx32$–92%) and tabanid flies ($d^*\approx32$–92%). Hence, in *P. aquatica* the values of d^* that can elicit positive polarotaxis are similar to that of dragonflies. The degree of polarization of water-reflected light is maximal at the Brewster angle, when the reflected light beam is perpendicular to the refracted one ($\theta_{Brewster}\approx53$ deg for the water surface measured from the vertical). According to Gál et al. (2001), Bernáth et al. (2004) and Horváth and Csabai (2014), the degree of polarization reflected by dark waters from the Brewster angle can reach $d\approx80\%$, almost independent of the solar elevation and sky conditions (clear or cloudy). For bright waters, the maximum of *d* can drop to about 25%, thus it can be questionable whether these waters can be detected polarotactically by aquatic insects with polarization sensitivity thresholds higher than 25%. According to the relatively low threshold of polarization sensitivity in *P. aquatica* ($10.1\%<d^*<25.5\%$), we conclude that the water springtail is equipped with a highly water-sensitive sensory system.

Based on our results, the most attractive stimulus was 100% and horizontally polarized, the unpolarized light elicited moderate attraction and the least attractive was the 100% vertically polarized stimulus (Table 2). In experiment 5, compared with unpolarized light, springtails were attracted to horizontal polarization and avoided vertical polarization (Fig. 8). The transition angle α^* (from the horizontal) at which springtails equally preferred the 100% polarized and unpolarized ($d\approx0\%$) stimulus, was not 45 deg, but closer to 50 deg. This slightly asymmetrical reaction in experiment 5 could arise from the slight intensity differences between the 100% polarized and unpolarized stimuli. Similar asymmetry occurred in experiment 4, where various degrees of polarization were tested against unpolarized stimulus and the attraction to horizontally polarized light was stronger than the avoidance of vertically polarized light. The reason for this may also be the slight intensity difference between the polarized and unpolarized stimuli, but for an exact answer, an additional experiment should be performed with equal stimulus intensities.

In experiment 6, we demonstrated that testing many (100–250) Collembola specimens simultaneously was a sound method, because the springtails tested individually expressed the same reactions (Table 2) as their counterparts in simultaneous experiments conducted with multiple springtails (experiment 4: horizontal polarizer in S_1, vertical polarizer in S_1, polarizer in S_{16}, Fig. 7).

Since *P. aquatica* springtails have horizontal and vertical microvilli in their ventral eye region (Paulus, 1972) and in our present study they showed unambiguous polarotaxis, we propose that this species possesses a visual system that enables it to detect water by means of the horizontal polarization of water-reflected light, as is the case in many other polarotactic aquatic insect species (reviewed in Horváth and Csabai, 2014). Labhart (1988) demonstrated the presence of polarization opponent neurons which connect photoreceptors with orthogonal microvilli in crickets. We hypothesize a similar mechanism in *P. aquatica*, where the sensed contrast between the horizontal and vertical microvillar systems offers the ability to estimate the angle and degree of polarization of light: 100% horizontally polarized, unpolarized and 100% vertically polarized light are points along a contrast gradient which determines the attractiveness. Our results highly support this concept, especially those from experiment 3. The outcomes of experiments 2, 4, 5 and 6

do not really require the springtails to estimate or measure the degree of polarization. If *P. aquatica* was just phototactic and detected only horizontal polarization (possessing only one, horizontal microvilli arrangement in all ommatidia), the latter experiments could give similar results. At the same time, the distribution of springtails in experiment 3 would be expected to be symmetrical when the intensity ratio of the 100% polarized and unpolarized stimuli was $I_{pol}/I_{unpol}=1/2$. In reality, the springtails preferred the 100% horizontally polarized light against unpolarized light even if the intensity of the former was 10 times dimmer. Consequently, *P. aquatica* has the ability to estimate the degree of polarization. Obviously, our findings are valid only in the visible spectral range, since our setup was not able to produce ultraviolet light. The spectral sensitivity of *P. aquatica* has not been measured yet, but it is expected to have at least one peak in the visible spectral range.

Since the few (two in the dorsal eye region and six in the ventral one) ommatidia of *P. aquatica* possess relatively large opening angles (Shaller, 1972), the field of view of the ventral eye region is capable of detecting water surfaces, even if the insect is crawling on a raised surface with its head tipped down (Fig. 1). This anatomical feature allowed us to use light stimuli coming from above the horizon viewed by the tested springtails placed on the bottom of the choice box. The attraction to horizontally polarized light definitely serves as a water detection system and basically helps the springtails to stay in the immediate vicinity of water, since *P. aquatica* springtails usually do not leave their habitat. However, after dispersion by wind, springtails may utilize their polarization sensitivity for habitat seeking.

Unlike the ventral eye region, the upper two ommatidia composing the dorsal eye region have only vertical microvilli, and it is still to be studied whether the dorsal eye region of *P. aquatica* can or cannot exploit polarization information. It has been shown that *P. aquatica* and other Collembola species are able to orient and maintain a certain direction under natural and artificial radiance distributions (Verheijen and Brouwer, 1971; Hågvar, 2000; Manica et al., 2000). However, it has not been studied whether springtail navigation and orientation are also governed by skylight polarization.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Á.E., G.H., G.K. designed the experiments, Á.E., A.F. performed the experiments, Á.E. did the programming and analyzed the data. Á.E., A.F., G.H., G.K. wrote and revised the paper.

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