SOME NOTES ON THE MATING BEHAVIOUR OF THE HARVESTMAN *PAROLIGOLOPHUS AGRESTIS* (OPILIONES, PHALANGIIDAE)

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ABSTRACT
The mating behaviour, copulation and anatomy of the genitalia of *Paroligolophus agrestis* (Meade, 1855) are analysed and depicted using a series of photographs made by Jürgen Peters. Species-specific adaptations of the female sternum and top of the genital operculum in *P. agrestis* may enable intromission of the penis while the operculum of the female remains closed. It is suggested the particularly long penis in *Paroligolophus agrestis* is an evolutionary attempt to take the sperm much further inside the ovipositor, closer to the eggs.
Key words: copulation, mating behaviour, mating success, Opiliones, *Paroligolophus agrestis*

On his website Insektenfotos.de webmaster Jürgen Peters presented a remarkable series of images depicting the mating ritual of *Paroligolophus agrestis* (Meade, 1855). A couple of this harvestman species was caught in the act of mating, sitting head to head on a rotting banana. The banana fruit was placed at eye level in a tree in his garden, with the purpose of attracting all kinds of invertebrates. Several species of harvestman, including *P. agrestis*, had been visiting the site for some nights, feeding on the decomposing banana. Luckily the harvestman couple was sitting in such a way that it could be observed and photographed from aside. This resulted in a sequence of eleven pictures, superbly revealing the mating procedure.

Since little attention has been paid to the study of most aspects of harvestman mating strategies, in this contribution I will attempt an analysis of the event. Interestingly, in the course of studying the pictures, some species-specific morphological features of both the male and female of *P. agrestis* seemed to literally fit in perfectly with the observations.

INTRODUCTION
Paroligolophus agrestis is a medium-sized, short-legged harvestman belonging to the subfamily Oligolophinae of the Phalangiidae. Identification of the species is quite easy since the yellowish ocularium has no tubercles, just a few tiny hairs. Most other members of the European Oligolophinae have two rows of more or less distinct tubercles on the ocularium. Near the front margin of the prosoma there is a group of three denticles, called the trident. Most specimens of *P. agrestis* have an indistinct central marking, with light and dark dots, often with vivid flushes of orange, reddish brown and yellow. Another characteristic in the field is a light yellowish to orange brown midline. Generally the males are darker and smaller.

This attractive species has an atlantic distribution, being found in Sweden, Norway, Denmark, Germany, Poland, the Netherlands, Belgium, Great-Britain, Ireland, France and Northern Spain. In the Netherlands and in large parts of the lower regions of Germany it is a common harvestman species, occurring in woodlands, as well as in manmade environments such as parks and gardens (Wijnhoven 2005, Blick & Komposch 2004). In the mentioned garden of Jürgen Peters *P. agrestis* is a very common species.

Adults occur from the beginning of July until February, the period of highest activity being late autumn to early winter. The pictures were taken on the evening of January 19, 2008, between 20.03 and 20.08 hour, in Borgholzhausen, Ostwestfalen, Germany (MTB 3815). From this it can be concluded the species not just stays alive, but is sexually active in winter as well. So egg deposition probably also occurs during late winter.

METHODS
On his website Insektenfotos.de webmaster Jürgen Peters published a sequence of nine pictures depicting the mating of *P. agrestis*. They can be viewed at: http://insektenfotos.de/forum/thread.php?threadid=12376. He then kindly granted my request and sent to me the eleven original, high resolution digital images. From this sequence eight characteristic photos were selected: 1, 2, 3, 5, 6, 9, 10 and 11 (the other three did not give additional information). The photos were enlarged and printed. With a transparent overlay sheet the details were drawn out (fig. 4 a- h).

Also some male and female specimens, collected in the Netherlands, were dissected to study the dimensions, positioning and appearance of the structures involved in the mating process.

SEXUAL MORPHOLOGY
Paroligolophus agrestis shows some unusual features, not found in any other harvestman species. The male genitalia and the female genital operculum show specific modifications that most probably are a consequence of some sexual selection process. For a better understanding of the function of these structures it may be illuminating to examine the mating strategy of this species.

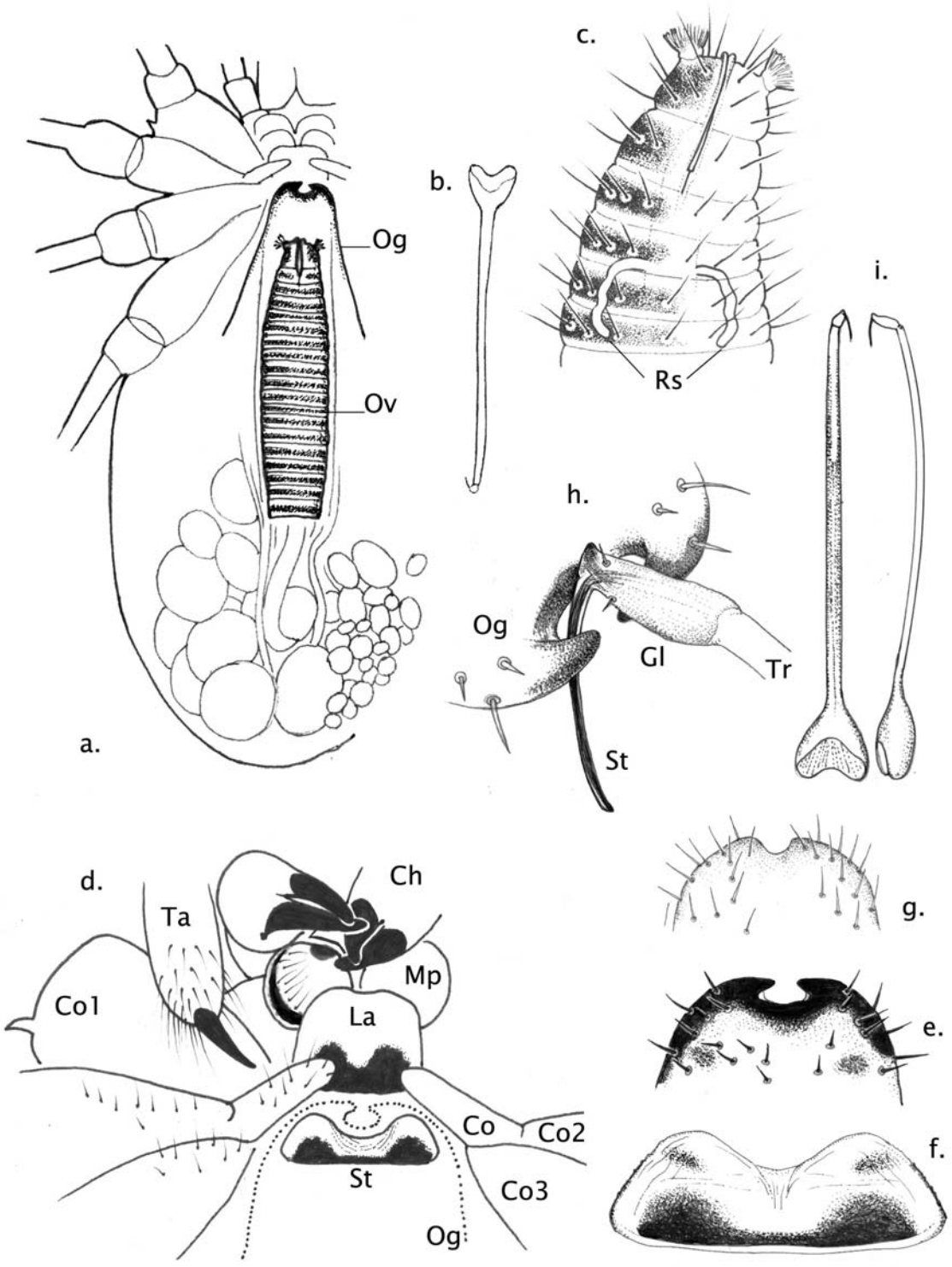

Figure 1. *Paroligolophus agrestis*, a. female, collected March 3 2008, Nijmegen, the Netherlands. The sternites have been removed to show the dimensions and position of the ovipositor (Ov) and the genital operculum (Og), b. male, penis, drawn on the same scale as the female in figure 1a to demonstrate the relative dimensions, c. Detail of the top of the ovipositor, with a pair of the seminal receptacles (Rs), left side with sclerotized parts drawn in, d. female, detail of the ventral area surrounding the genital operculum (Og, dotted line), Ch: chelicerae, Co: coxapophyses of second leg, Co1: Coxa of first leg, Co2: coxa of second leg, Co3: coxa of third leg, Og: genital operculum, La: labium, Mp: mouthparts, St: sternum, Ta: tarsus of the pedipalp, e. Top of the female genital operculum, f. Female sternum, g. Top the male genital operculum, h. Penis glans 'locking' onto the female genital operculum, Gl: Glans, Og: Female genital operculum, St: Stylus, Tr: Truncus, i. Male, penis, left ventral view, right lateral view. Figure d-f: sclerotized parts are drawn in black. All drawings H. Wijnhoven.

The truncus of the penis is extremely long and slender, parallel sided, flattened dorsoventrally and heavily sclerotized (fig. 1b, i). Conversely, the base of the truncus is abruptly broadening, containing a large concentration of internal muscles. The glans is quite small, as wide as the shaft, with a very large, rather robust, sclerotized stylus at the top (fig. 1h). The male genital operculum has an indistinct notch at the middle top section, not found in other Oligolophinae species (fig. 1g).

The genital operculum of the female has a characteristic triangular opening at the top (fig. 1a, e, d, h), the meaning of which is not yet fully understood. Martens (1978) assumes it may function as a means to guide the penis into the pregenital chamber.

Other details of the male and the female genitalia and structures presumably involved in the mating process are discussed below.

MATING BEHAVIOUR OF *PAROLIGOLOPHUS AGRESTIS*

In Opilionids of the Phalangiidae the copulation takes place in a face-to-face position. In several species some form of courtship may precede the actual copulation ritual, like the male tapping the female's back with the pedipalps or with the legs. Also mutual leg tapping or rubbing has been reported (Machado & Macías-Ordoñes 2007). In the case of *P. agrestis* we do not know this, as the photograph (fig. 2) was taken when the male and female already had grasped each other. It can be seen the male has taken hold of the bases of the female's first legs with his pedipalps. The tarsi of his first pair of legs are in full contact with the female's back. His second and fourth pairs of legs are outstretched to stabilize the position of the body. The third leg is hooked on to her second leg, and the tarsi at the same time grasp her third leg.

A similar position of the bodies, legs and pedipalps can be recognised on the series of images to be discussed here, although in this case the female seems to have lost at least the second and fourth left leg, thus the grasping manner somewhat differs (fig. 3). As the whole sequence of eleven images depicts the mating pair from a lateral viewpoint, the position of respectively the left male and right female legs can not be judged. The male seems to regularly touch the extruding tract with the tarsus of his third right legs (fig. 4a-f, but only drawn in fig. 4a and 4d). At an early stage (especially fig. 4a to 4c) both male and female palpal tarsi appear to touch the male tract. Also the inflated mouthparts of the female are visible in most pictures (fig.4c to 4h) indicating they may in some manner be involved too.

The genital operculum of the male then is fully opened and the male reproductive tube starts to extrude. This is achieved by a combination of muscle contractions and hydraulic pressure of the haemolymph. The whitish, somewhat translucent tube, supporting and guiding the slender penis shaft, makes full contact with the female genital operculum (fig. 4d). Remarkably, during the whole process of copulation, the genital plate of the female does not seem to open up much, if at all. The muscular and strongly broadened base of the shaft can be recognised through the transparent membrane (fig. 4d to 4g), indicating the penis eventually is inserted almost to its full length.

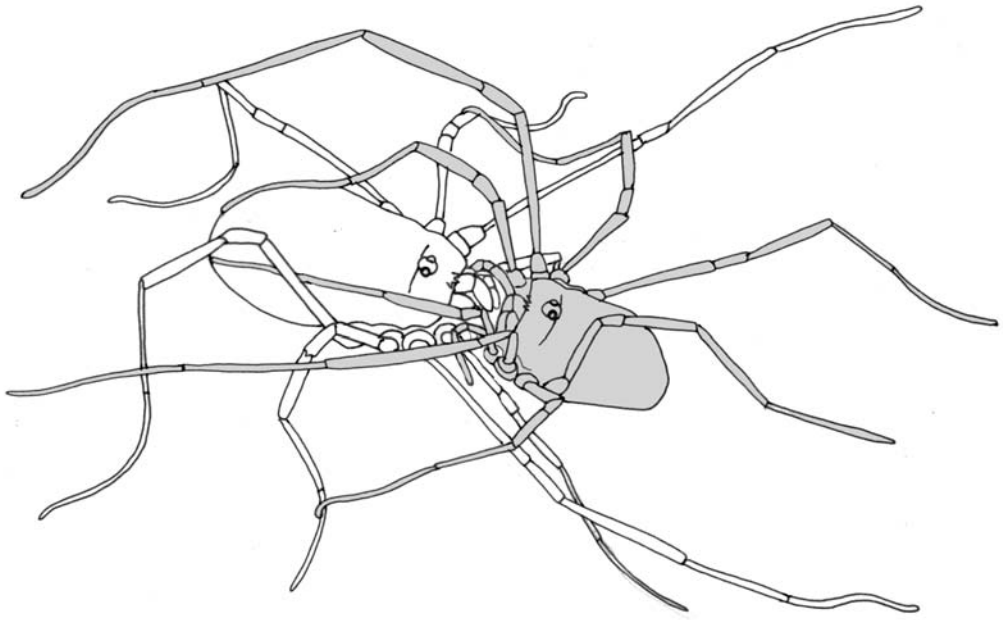

Figure 2. *Paroligolophus agrestis*, mating couple. Drawing based on a photo by Jürgen Peters, December 13 2007, Borgholzhausen, Ostwestfalen, Germany. Male right (in grey), female left.

Figure 3. *Paroligolophus agrestis*, mating couple. Photo number 4, Jürgen Peters. January 19 2008, Borgholzhausen, Ostwestfalen, Germany. Male left, female right.

Finally the male pregenital lumen as well as the penis is withdrawn (fig. 4g, h). Most likely this is accomplished by the reverse process: activity of muscles attached to the walls of the pregenital chamber together with reduced hydraulic pressure of the haemolymph, cause the male tract to deflate. The observed mating process lasted for about five minutes.

REMARKS ON THE MATING PROCESS

As in harvestmen both males and females are competent of mating several times with different partners, a considerable degree of sexual competition has to be involved. This will imply selective pressure on mating strategies. The interactions between both sexes may result in species-specific adaptations in behaviour as well as in morphological details. For example males often have evolved modified pedipalps so as to sustain a strong grasp of the female. For this purpose the male palpal tarsus of *P. agrestis* is provided ventrally of rows of small spines. This is a common feature in most Phalangiidae males, demonstrating that males of these species generally must have a similar manner of grasping the females by the first legs. An example of a unique species-specific morphological detail, probably pointing towards a somewhat different mating strategy, can be found in the male chelicerae of the related *Oligolophus hanseni* (Kraepelin, 1896).

In all harvestman species of the suborder Eupnoi (to which the Phalangiidae-Oligolophinae belong) the spermatozoa lack a flagellum and they are therefore unable to move (Machado, G. & R. Macías-Ordoñes 2007). Generally it is thought the sperm cells are being deployed near or inside the so called seminal receptacles. These are a pair of small tube to pocket shaped structures near the tip of the female ovipositor (fig. 1c). It is thought the sperm cells can be stored inside them for some time. On their way out the eggs pass these receptacles and are fertilized just before they are inserted into a suitable substrate, for example the soil or crevices in rotting tree truncks.

So it is quite crucial to keep in mind that an accomplished copulation does not necessarily imply fertilization. To our eyes it may seem in harvestmen the male forces copulation by strongly grasping the female. Yet the female can reject intromission of the penis by keeping the genital operculum closed. Furthermore the female can also prevent a successful copulation by keeping the apical lobes of the ovipositor closed, so the penis can not reach the seminal receptacles. And even if intromission has been accepted by the female and the male has delivered the sperm, she may have some control over their fate (Eberhard 2000).

In *P. agrestis* the shape of the penis in cross-section coincides well with the ‘negative’ shape of the triangular opening of the female genital operculum, suggesting indeed the penis is guided through it, as Martens (1978) supposed. The observed closed (or nearly closed) genital operculum of the female during mating supports this assumption. From the pictures it appears, the stylus and glans of the penis are ‘locked’ on to the structure at the margin of the female genital operculum (fig.1h). This would also explain the function of the robust stylus,

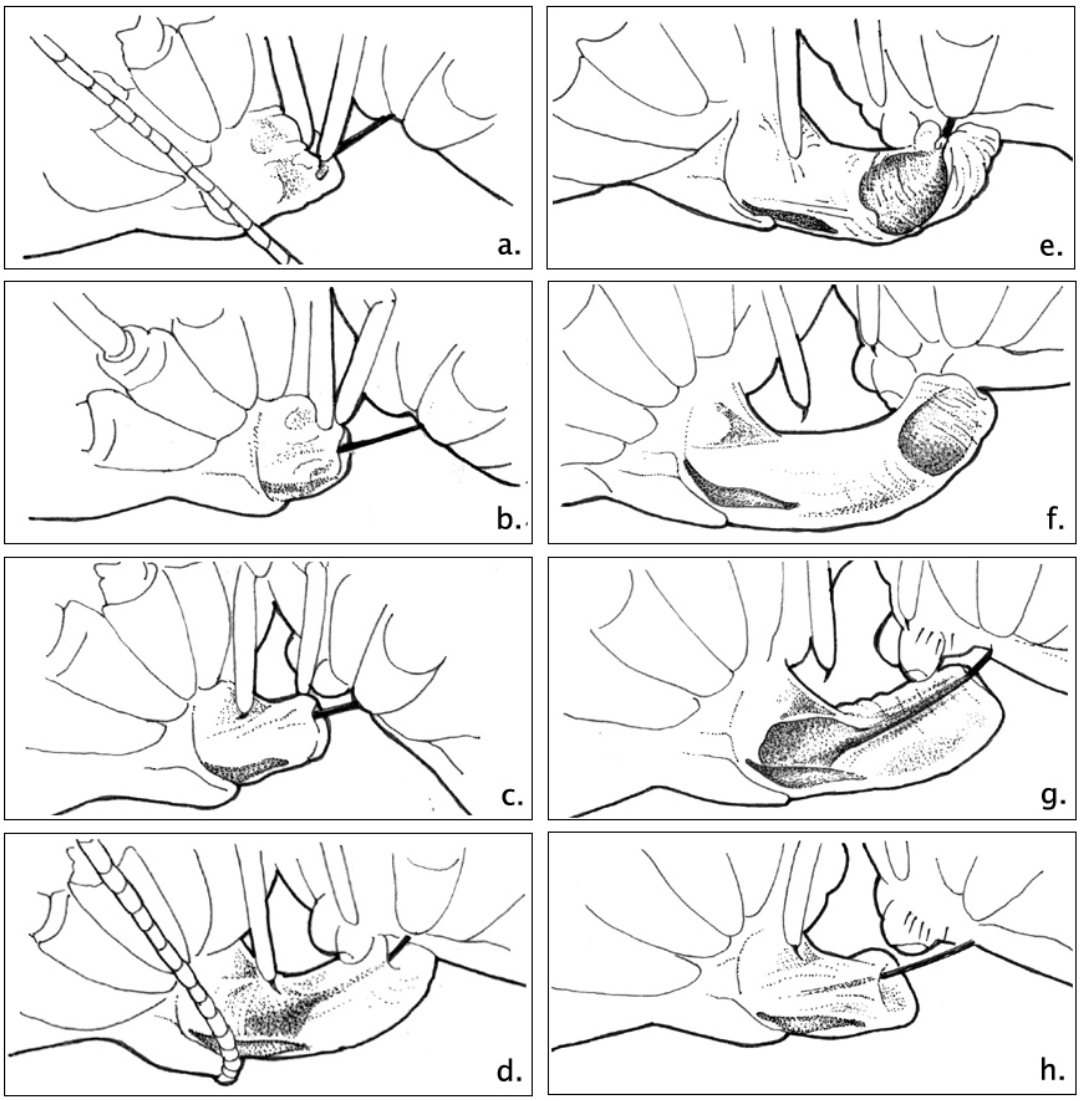

Figure 4. a.- h. *Paroligolophus agrestis*, mating couple, 8 details after a photo sequence of 11 photos by Jürgen Peters. January 19 2008, Borgholzhausen, Ostwestfalen, Germany. Selected photos: 1, 2, 3, 5, 6, 9, 10 and 11. Male left, female right.

the apical position of the sensory hairs of the glans, the sclerotized top of the glans as well as the heavily sclerotized female opening, which is acting as a lever joint once the penis glans has passed through it. All of these structures need to be rigid and strong.

Another morphological adaptation that seems to coincide with the observation of a closed female genital operculum during mating, in my opinion is the shape of the sternum, which is positioned just underneath the top of the female genital operculum. This sternum has a sclerotized, semicircular depression (fig 1d, 1f). This too may enable the penis to pass through even if the genital operculum is closed.

Possibly at this initial mating stage the female gathers some particular cues (chemical or tactile signals or both) to evaluate her potential mate. It could be rewarding to look for sensory organs of some kind at the top of the female genital operculum. Also the female's mouthparts, chelicerae, the coxapophyses of the second pair of legs as well as the labium may somehow be involved in the female's evaluation of her mate. These structures are all concentrated near the top region of the genital operculum (fig.1d). They may also be engaged in guiding the penis or, conversely, in rejecting intromission in case of a negative evaluation.

No other Oligolophinae species does have a penis that slender as to physically be capable of entering a locked female genital operculum. So obviously by keeping the genital operculum (nearly) closed, the female can efficiently prevent males of other species to try mate with her.

Once the penis glans has 'matched' with the female genital operculum and the female presumably has 'recognized' and accepted her mate (fig. 4a, b), the male reproductive tract is fully extruded. Guided by the fixed position of the penis it now can be forced out to make full contact with the female genital operculum. Subsequently the male reproductive tract itself enables the penis to be extruded much further. At the base of the

extruded tube a pair of sclerotized structures is present. They perform as an extension of the male genital operculum, supporting the penis base (see for example fig. 4g) on its way out.

Figure 4e seems to be quite crucial. It apparently depicts the actual insemination. The penis is extruded to its maximum, the base of the broadened shaft is almost in contact with the female genital operculum. At this point the male reproductive tube is not transparent any more, but whitish, indicating that the propulsive organ also has been forced out. The slight distortions as well as the somewhat wrinkled surface structure of the male tract demonstrate the hydraulic pressure by now has reached a maximum and the strongly muscular propulsive organ is forcing the semen through the ejaculatory duct.

Now the typical robust, almost cylindrical shape of the base of the penis truncus can be valued. To begin with, the muscles have to be concentrated at the extreme base to enable the slender shaft to be inserted as far inside as possible during copulation. Secondly at a strongly broadened base a large amount of intrinsic muscles can be attached, enabling the penis to be manoeuvred with more precision inside the female tract. Finally the base itself is a voluminous structure, working like a piston, so more hydraulic pressure can be exerted on it by the walls of the male reproductive tract.

CONCLUSIVE REMARKS

In the sequence of pictures taken by Jürgen Peters a few particularly rare circumstances luckily came together, offering the opportunity to analyse the mating process in detail. Firstly the harvestman couple could be photographed from a lateral viewpoint. Also, due to the dark background the male reproductive organs appeared somewhat translucent, revealing the internal structures. Finally, except for the very beginning, the complete mating process was depicted, and these photos were of excellent quality.

Special adaptations of the female sternum and top of the genital operculum in *P. agrestis* apparently enable intromission of the penis while the operculum of the female remains closed.

In my opinion the photos strongly suggest the male does not put the sperm into the seminal receptacles, near the top of the ovipositor, but much further inside. Maybe the particularly long penis in *Paroligolophus agrestis* is an evolutionary attempt to take the sperm much further inside the ovipositor, closer to the eggs. The idea is that the male's gametes closest to the eggs, will have the best prospect of actually fertilizing them. This, however, remains to be confirmed.

ACKNOWLEDGMENTS

I am very grateful to Jürgen Peters (Insektenfotos.de) who kindly has sent to me the original digital images of *Paroligolophus agrestis* and provided me of additional information.

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SAMENVATTING

Op de website Insektenfotos.de publiceerde webmaster Jürgen Peters een opmerkelijke serie van 11 foto's van een parend stel hooiwagens *Paroligolophus agrestis* (http://insektenfotos.de/forum/thread.php?threadid=12376). De paring is van een zijdelings standpunt vastgelegd. Vanwege de donkere achtergrond zijn de mannelijke geslachtsorganen iets doorschijnend, zodat de inwendige structuren zichtbaar worden. Uit deze reeks zijn acht karakteristieke foto's gekozen met de bedoeling te analyseren hoe de paring in zijn werk gaat (fig. 4).
Deze waarnemingen worden in verband gebracht met een aantal soortspecifieke, morfologische eigenaardigheden van *P. agrestis*. De penis bijvoorbeeld is uitzonderlijk lang en smal, met een zeer brede, van spieren voorziene basis (fig. 1b, h, i). Het vrouwtje heeft aan de top van de genitale opening een kenmerkende inkeping (fig. 1a, d, e). De fotoreeks lijkt aan te tonen dat de penis in deze uitholling wordt "vastgehaakt". Door deze gefixeerde positie kan de penis tot aan de basis van de schacht in de legbuis doordringen. De genitale opening van het vrouwtje kan hierbij vrijwel gesloten blijven. Meestal wordt er van uitgegaan dat de spermacellen in speciale orgaantjes aan de top van de legbuis worden afgezet (fig. 1c), maar de waarnemingen lijken er eerder op te wijzen dat de zaadcellen zo dicht mogelijk in de buurt van de eitjes worden afgezet, zodat ze meer kans maken deze te bevruchten. Aangezien beide seksen meerdere keren met verschillende partners kunnen paren, zijn deze aanpassingen in anatomie en gedrag mogelijk geëvolueerd onder invloed van seksuele competitie.