Sci Nat (2016) 103:11
DOI 10.1007/s00114-016-1337-4

SHORT COMMUNICATION

Penis morphology in a Burmese amber harvestman

Jason A. Dunlop[1] · **Paul A. Selden**[2,3] · **Gonzalo Giribet**[4]

Received: 7 December 2015 / Revised: 17 January 2016 / Accepted: 18 January 2016

Abstract A unique specimen of the fossil harvestman *Halitherses grimaldii* Giribet and Dunlop, 2005 (Arachnida: Opiliones) from the Cretaceous (ca. 99 Ma) Burmese amber of Myanmar reveals a fully extended penis. This is the first record of a male copulatory organ of this nature preserved in amber and is of special importance due to the age of the deposit. The penis has a slender, distally flattened truncus, a spatulate heart-shaped glans and a short distal stylus, twisted at the tip. In living harvestmen, the penis yields crucial characters for their systematics. Male genital morphology in *H. grimaldii* appears to be unique among the wider Dyspnoi clade to which this fossil belongs. The large eyes in the fossil differ markedly from other members of the subfamily Ortholasmatinae to which *H. grimaldii* was originally referred. Based on recent data, it has been argued that large eyes may be plesiomorphic for Palpatores (i.e. the suborders Eupnoi and Dyspnoi), potentially rendering this character plesiomorphic for the fossil too. Thus, the unique structure of the penis seen here, and the probable lack of diaphanous teeth, present in all other extant non-acropsopilionid Dyspnoi, suggest that *H. grimaldii* represents a new, extinct family of large-eyed dyspnoid harvestmen, Halithersidae fam. nov.; a higher taxon in amber diagnosed here on both somatic and genital characters.

Keywords Arachnida · Opiliones · Male genitalia · Systematics · Amber · Myanmar

Communicated by: Sven Thatje

✉ Jason A. Dunlop
jason.dunlop@mfn-berlin.de

[1] Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Invalidenstrasse 43, 10115 Berlin, Germany

[2] Department of Geology and Paleontological Institute, University of Kansas, 1475 Jayhawk Boulevard, Lawrence, KS 66045, USA

[3] Department of Earth Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK

[4] Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

Introduction

Harvestmen (Arachnida: Opiliones) characteristically have eversible genitalia (Macías-Ordóñez et al. 2010), a rare condition among arachnids. In the suborder Cyphophthalmi, the male organ deposits a spermatophore (Karaman 2005) and is usually referred to as a spermatopositor. In the Phalangida suborders (Eupnoi, Dyspnoi and Laniatores), the male has an intromittent organ, or penis, which is used for direct sperm transfer via copulation. The penis is usually held within the body when not in use. This limits the chances of finding this structure in fossils, even well-preserved ones. Fossil harvestmen are rare (38 species described to date). Most Palaeozoic and non-amber Mesozoic fossils are known from one or only a few specimens. Amber specimens can be more common. In particular, some Baltic amber species are known from multiple specimens which can potentially be assigned to males and/or females based on secondary sexual characters such as modifications of the male chelicerae.

Primary male genital morphology (the penis) has been observed in three non-amber Devonian (Dunlop et al. 2003, 2004), Carboniferous (Garwood et al. 2014) and Jurassic (Giribet et al. 2012) examples. However, most fossil harvestmen originate from Mesozoic and Cenozoic ambers. Many of these look very similar to living genera, although several species have been

Published online: 28 January 2016

found which do not appear to have a modern equivalent (Giribet and Dunlop 2005; Dunlop and Mammitzsch 2010; Dunlop et al. 2012). In fact, one of the biggest challenges in reconciling studies of fossil and Recent harvestmen is the absence of male genital characters in most of the fossils, given that penis morphology is of considerable significance for the systematics of both extant species and the higher groups to which they belong (e.g. Martens 1976, 1986). As has been demonstrated for insects (Perreau and Tafforeau 2011), future applications of microtomography may reduce the gap between fossil and living harvestman species through being able to resolve internal genital structures too.

Here, we report a remarkable example of a fossil harvestman from the Cretaceous (ca. 99 Ma) Burmese amber of Myanmar assignable to *Halitherses grimaldii* Giribet and Dunlop, 2005. In this new inclusion, the penis is uniquely preserved as a fully extended and externally visible structure (Figs. 1 and 2). Furthermore, details of its morphology differ from the penis structure of other members of the wider Dyspnoi clade to which this fossil belongs. This is the first time the penis has been seen in an amber harvestman and allows valuable insights into the relationships of an extinct genus, previously difficult to place at family level.

Material and methods

The new specimen comes from the Jörg Wunderlich collection and bears the repository number F2840/BU/CJW. It will be transferred at a later date to the Senckenberg Institute

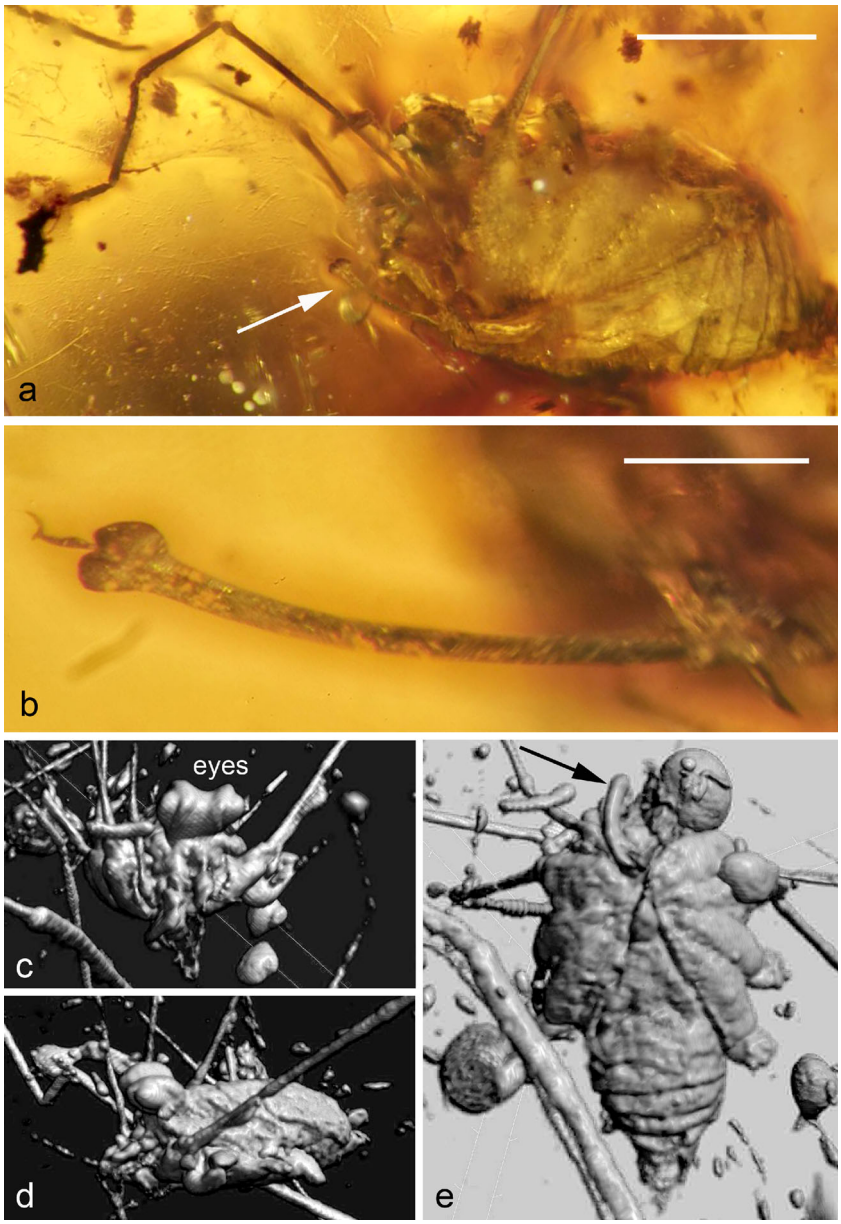

Fig. 1 A fossil harvestman (Arachnida, Opiliones), *Halitherses grimaldii* Giribet and Dunlop, 2005, from the Cretaceous Burmese amber of Myanmar. **a** Lateral overview showing the penis (*arrowed*). *Scale bar* 1.0 mm. **b** Details of penis morphology. *Scale bar* 0.2 mm. **c** Computed microtomography reconstruction in frontal view showing the caddid-like eye tubercle. **d** Computed microtomography reconstruction in lateral view. **e** Reconstruction in ventral view showing the penis emerging from beneath the genital operculum (*arrowed*) and the adjacent disposition of the leg coxae

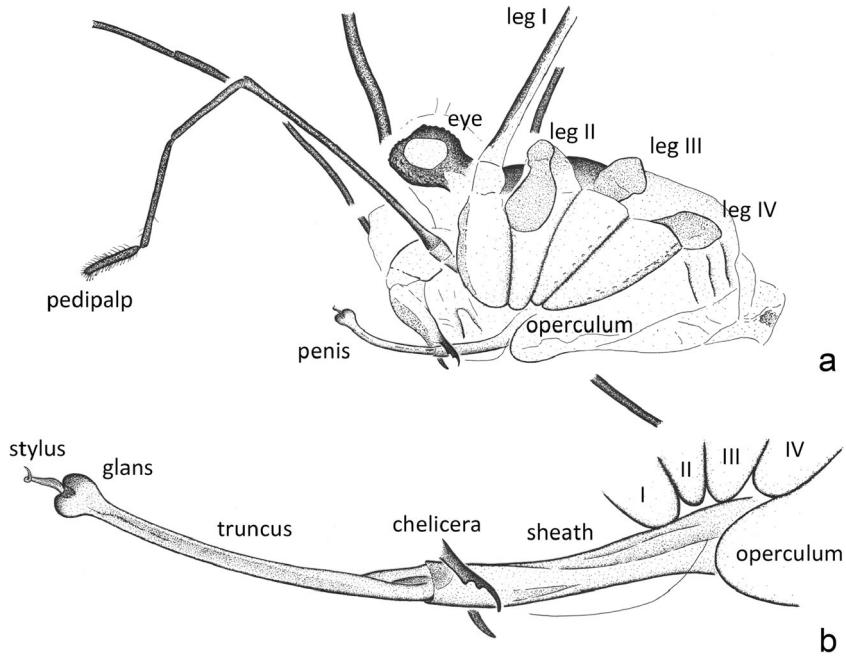

Fig. 2 Interpretative drawings of the specimen shown in Fig 1. **a** Lateral overview. **b** Details of the penis morphology

Frankfurt/Main, or one of its satellite institutions (J. Wunderlich, personal communication). Burmese amber originates from the Hukawng Valley in the northern state of Kachin, Myanmar. It is thought to have been deposited in a tropical forest environment and was recently dated at Late Cretaceous (earliest Cenomanian), or about 98.79 ± 0.62 Ma (Shi et al. 2012). For a recent overview of this amber deposit and its geological setting, see Ross et al. (2010). The inclusion was studied and drawn under a Leica MZ12.5 stereomicroscope with a *camera lucida* attachment. It was photographed using a Canon 5D MkIII camera attached to the microscope, captured using DSLR Assistant (www.dslrassistant.com) onto a MacBook Pro computer, and manipulated (including image stacking) in Adobe Photoshop CC (www.adobe.com). All measurements are in millimeters.

Tomography

Additionally, computed microtomography was applied using a SkyScan 1173 μCT scanner (Bruker MicroCT, Kontich, Belgium) equipped with a Hamamatsu 130/300 tungsten X-ray source and a FlatPanel Sensor camera detector with 2240 × 2240 pixels. Scanning parameters were as follows: source voltage = 70 kV, source current = 95 μA, voxel size = 6.04 μm, exposure time = 1000 ms, frames averaged = 7, frames acquired over 180° = 2399, filter = no, binning = no, flat field correction = activated and scanning time = 04:41:58. Reconstruction of the two-dimensional (2D) projection images into a 3D volumetric image stack was performed using the software NRecon 1.6.6.0 (Bruker MicroCT, Kontich, Belgium). While this proved helpful in resolving some ventral aspects of the body (Fig. 1e), we were unable to resolve additional characters from the penis beyond those visible using traditional light microscopy (Fig. 1b).

Results

Systematic palaeontology

Opiliones Sundevall, 1833

Suborder Dyspnoi Hansen & Sørensen, 1904

Halithersidae fam. nov.

Type and only genus: *Halitherses* Giribet and Dunlop, 2005

H. grimaldii Giribet and Dunlop, 2005

Figs. 1 and 2.

Material F2840/BU/CJW. Burmese amber from Myanmar. Late Cretaceous: Cenomanian.

Diagnosis of the new family Dyspnoids with ornamented carapace, dominated by massive eyes on a large, bilobed, forward-projecting ocularium; albeit without a median hood process or "troguloid facies" as per ortholasmatines (Figs. 1a, c, d, 2a). With a free metapeltidium (erroneously given as a fused meso- and metapeltidium in the original description) and scutum parvum. Chelicerae moderately sized and robust, without apparent diaphanous teeth. Pedipalps elongate and mostly cylindrical, with glandular setae on the tarsus and tibia; tarsus shorter than tibia. Legs extremely long and slender, more than 10 times body length. Coxa IV oriented posteriorly, forming a 30° angle with body (Fig. 1e). *Pars distalis* of penis forms spatulate, heart-shaped glans bearing V-shaped anterior invagination from which a short, tapering and distally twisted stylus emerges (Figs. 1b, 2b).

Description Largely complete harvestman, best seen in ventro-lateral view. Body length ca. 2.3. Ocularium large (diameter ca. 0.4), bearing equally large eyes and with strongly tuberculate rim. Chelicerae moderately sized (length ca. 0.9); free finger with two large teeth; without apparent diaphanous teeth. Pedipalps long (ca. 2.8), slender; femur much longer than other articles. Palpal tarsus quite short; bearing clavate glandular setae, but lacking terminal palpal claw. Legs mostly disarticulated but, where present, highly elongate and slender. Dorsal surface of body largely equivocal. Opisthosomal segmentation visible posteriorly, but ventral surface dominated by the four leg coxae plus tongue-shaped genital operculum from beneath which a styliform penis (length ca. 1.5) emerges.

Penis slender, elongate, curving slightly upwards. Proximal region (*pars basalis*) consists of long truncus (length 1.35), basal 0.6 of which enveloped by folded cuticular sheath. Truncus more flattened than rounded in cross section, especially distally; dark line within distal region could represent muscle tendon. Distal region (*pars distalis*) consists of heart-shaped glans, length 0.1, flattened like adjacent truncus and thus spatulate, darker anteriorly, with V-shaped anterior invagination. Glans lacks obvious ornament (hooks, setae, etc.). Apical stylus, length ca. 0.1, emerges from invagination of glans. Stylus cuticle thin, basally slightly wider, tapering distally. Terminal tip either asymmetrically bifurcate or, more likely, curved and twisted to the right.

Discussion

The large, ornamented eye tubercle, the slender legs and pedipalps, and the clavate setae on the pedipalp tip (Figs. 1a, c, d, 2a) all support the referral of our new inclusion to the previously described Burmese amber species *H. grimaldii*. These clavate setae and the absence of a distal palpal claw again confirm referral to the suborder Dyspnoi, as per the original description, although unlike the non-acropsopilionid Dyspnoi, *H. grimaldii* seems to lack diaphanous cheliceral teeth. The genital characters recovered here and the lack of the diaphanous cheliceral teeth are of particular significance given uncertainties in the original familial placement of this species. Based on the initial phylogenetic analysis, Giribet and Dunlop (2005) referred *H. grimaldii* to the subfamily Ortholasmatinae of the dyspnoid family Nemastomatidae. However, this placement was admittedly tenuous and based mostly on biogeographical grounds; whereby this subfamily also occurs commonly in Southeast Asia today (e.g. Schwendinger and Gruber 1992). The position of *H. grimaldii* was discussed by Shear (2010; see also below), who suggested it might represent a new family. Since the original description, *H. grimaldii* has been included in three combined analyses of molecules and morphology (Garwood et al. 2011, 2014; Sharma and Giribet 2014), but its position continued to be largely unresolved or unsupported. Kury (2013) placed the fossil under Dyspnoi *incertae sedis*, while fossils were not included in a recent catalogue of Dyspnoi by Schönhofer (2013).

As discussed by Shear (2010, p. 13), *H. grimaldii* expresses a "…very large caddid-like eye tubercle with a median depression, that resembles no known nemastomatid". The large-eyed family Caddidae belongs to another suborder, Eupnoi. This resemblance between *H. grimaldii* and Caddidae was also noted by Giribet and Dunlop (2005, p. 1010), but at that time no caddid-like animal was known from the suborder Dyspnoi, and the other dyspnoan characters (see above) outweighed these caddid type of eyes. However, during a recent analysis of Caddoidea by Groh and Giribet (2015), members of the caddoid subfamily Acropsopilioninae were transferred from Eupnoi to Dyspnoi as the sister-group to all other Dyspnoi. This important taxonomic change now gives us precedent for there being large-eyed dyspnoid harvestmen which lack diaphanous cheliceral teeth as well. It further forces the Palpatores ancestor (i.e. the common ancestor of Eupnoi and Dyspnoi) to present large, caddid-like eyes. This distinctive eye morphology, also seen in the amber fossil, may thus now be considered a plesiomorphic trait for Palpatores.

In summary, the unusually large eyes in the Burmese amber harvestman *H. grimaldii* (Fig. 1c–d) may actually reflect a plesiomorphic character state for Dyspnoi. As per Shear's (2010) comments, large eyes also argue against affinities with the family Nemastomatidae. In fact the penis of *H. grimaldii*—revealed here for the first time in this remarkable amber inclusion—expresses a unique morphology within Dyspnoi, namely the spatulate, heart-shaped glans and the twisted apical stylus (Figs. 1b, 2b). This genital morphology in the fossil is probably apomorphic and comparative images for other (extant) dyspnoid harvestman families can be found in, e.g. Gruber (2007), or in Martens's (1983) *Sabacon* study and Schönhofer and Martens's (2012) overview of male genitalia in Nemastomatidae. Thus, the penis and the lack of diaphanous cheliceral teeth enable us to follow Shear's (2010) proposal and define a new fossil family, Halithersidae, as above. For the first time with an amber harvestman, we can define a higher taxon on a combination of somatic and male genital characters; as would be usual in the systematics of living taxa.

Acknowledgements We thank Jörg Wunderlich (Hirschberg) for making material from his collection available and Jenn Lenihan (Harvard) for producing the micro-CT images. Five anonymous reviewers provided constructive criticism.

References

Dunlop JA, Mammitzsch L (2010) A new genus and species of harvestman from Baltic amber. Palaeodiversity 3:23–32

Dunlop JA, Bartel C, Mitov PG (2012) An enigmatic spiny harvestman from Baltic amber. Foss Rec 15:91–101

Dunlop JA, Anderson LI, Kerp H, Hass H (2003) Preserved organs of Devonian harvestmen. Nature 425:916

Dunlop JA, Anderson LI, Kerp H, Hass H (2004) A harvestman (Arachnida: Opiliones) from the Early Devonian Rhynie cherts, Aberdeenshire, Scotland. Trans R Soc Edinburgh, Earth Sci 94: 341–354

Garwood RJ, Dunlop JA, Giribet G, Sutton MD (2011) Anatomically modern Carboniferous harvestmen demonstrate early cladogenesis and stasis in Opiliones. Nat Comm 2:444

Garwood RJ, Sharma PP, Dunlop JA, Giribet G (2014) A new stem-group Palaeozoic harvestman revealed through integration of phylogenetics and development. Curr Biol 24:1–7

Giribet G, Dunlop JA (2005) First identifiable Mesozoic harvestman (Opiliones: Dyspnoi) from Cretaceous Burmese amber. Proc R Soc B 272:1007–1013

Giribet G, Tourinho AL, Shih C, Ren D (2012) An exquisitely preserved harvestman (Arthropoda, Arachnida, Opiliones) from the Middle Jurassic of China. Org Div Evol 12:51–56

Groh S, Giribet G (2015) Polyphyly of Caddoidea, reinstatement of the family Acropsopilionidae in Dyspnoi, and a revised classification system of Palpatores (Arachnida, Opiliones). Cladistics 31:277–290

Gruber J (2007) Taxonomy: Dyspnoi. In: Pinto da Rocha R, Machado G, Giribet G (eds) Harvestmen. The biology of Opiliones. Harvard University Press, Cambridge, pp 131–159

Karaman I (2005) Evidence of spermatophores in Cyphophthalmi (Arachnida, Opiliones). Rev suisse Zool 112:3–11

Kury AB (2013) Order Opiliones Sundevall, 1833. Zootaxa 3703:27–33

Macías-Ordóñez R, Machado G, Pérez-González A, Shultz JW (2010) Genitalic evolution in Opiliones. In: Leonard J, Córdoba-Aguilar A (eds) The evolution of primary sexual characters in animals. Oxford University Press, Oxford, pp 285–306

Martens J (1976) Genitalmorphologie, System und Phylogenie der Weberknechte (Arachnida, Opiliones). Entomol German 3:51–68

Martens J (1983) Europäische Arten der Gattung *Sabacon* Simon 1879 (Arachnida: Opiliones: Sabaconidae). Senckenbergiana Biol 63: 265–296

Martens J (1986) Die Grossgliederung der Opiliones und die Evolution der Ordnung (Arachnida). Actas X Congr Int Arachnol, Jaca 1:289–310

Perreau M, Tafforeau P (2011) Virtual dissection using phase-contrast X-ray synchrotron microtomography: reducing the gap between fossils and extant species. Syst Ent 36:573–580

Ross A, Mellish C, York P, Crighton B (2010) Burmese amber. In: Penney D (ed) Biodiversity of fossil in amber from the major world deposits. Siri Scientific Press, Manchester, pp 208–235

Schönhofer AL (2013) A taxonomic catalogue of the Dyspnoi Hansen and Sørensen, 1904 (Arachnida: Opiliones). Zootaxa 3679:1–68

Schönhofer AL, Martens J (2012) The enigmatic Alpine opilionid *Saccarella schilleri* gen. n., sp. n. (Arachnida: Nemastomatidae)—isolated systematic placement inferred from comparative genital morphology. Org Div Evol 12:409–419

Schwendinger PJ, Gruber J (1992) A new *Dendrolasma* (Opiliones, Nemastomatidae) from Thailand. Bull Br arachnol Soc 9:57–60

Sharma PP, Giribet G (2014) A revised dated phylogeny of the arachnid order Opiliones. Front Genet 5:255

Shear WA (2010) New species and records of ortholasmatine harvestmen from México, Honduras, and the western United States (Opiliones, Nemastomatidae, Ortholasmatinae). ZooKeys 52:9–45

Shi G, Grimaldi DA, Harlow GE, Wang J, Wang J, Yang M, Lei W, Li Q, Li X (2012) Age constraint on Burmese amber based on U–Pb dating of zircons. Cret Res 37:155–163