Arthropod Structure & Development xxx (2016) 1–14

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

The water-repellent cerotegument of whip-spiders (Arachnida: Amblypygi)

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ARTICLE INFO

Article history:
Received 7 June 2016
Received in revised form
26 August 2016
Accepted 11 October 2016
Available online xxx

Keywords:
Anti-wetting
Surface coating
Plastron
Colloid
Arachnida
Amblypygi

ABSTRACT

The cuticle of arthropods is usually composed of layers of a chitin-protein-microcomposite, a proteinaceous epicuticle and a thin lipid coating. However, in some instances a thick cement layer (cerotegument) covers the cuticle and may produce elaborate microstructures. This has previously been described for millipedes and mites. Here we report the previously unknown presence of a superhydrophobic cerotegument in whip-spiders (Ambypygi) and reveal its variation in ultrastructure and water-repellence between species. We discuss the relevance of found micro-morphological and physical characters for taxonomy and phylogenetics of this group, and the potential biological functions.

1. Introduction

Due to its composite structure and stability the cuticle of arthropods is a highly versatile biological material that can form a huge variety of macro- and micro-structures. The cuticle is usually composed of numerous layers of a fibrous chitin-protein mixture in the endo-, meso- and exocuticle, and a thin protein-rich epicuticle that is often coated by a film of hydrocarbons (Vincent, 2002). Besides, in some instances a thick cement layer built after moulting is present, forming an elaborate surface structure. For such an additional layer of hardening secretion covering the epicuticle the term 'cerotegument' has been introduced. The term is derived from Latin *cera* (=wax) and *tegumentum* (=covering) (Maggenti et al., 2005), however, this does not implicate that cement layers named like this are composed of waxes. A cerotegument is present in millipedes (Adis et al., 1998) and two related orders of mites, the Sarcoptiformes and Trombidiformes (Alberti et al., 1981; Alberti and Coons, 1999). During studies on a small order of small to extraordinary large arachnids, the whip-spiders (Amblypygi) (Wolff et al., 2015b), we became aware of a powder-like coating frequently present on various body parts of these animals. The correlated occurrence of such a 'powder' and glandular openings has previously been remarked by Weygoldt (1996, 1998, 2000), but never been studied in detail.

Whip-spiders are regarded as sister group to whip-scorpions (Uropygi = Thelyphonidae + Schizomida) (Thorell, 1877; Pocock, 1893; Shultz, 1990, 2007; Garwood et al., 2016), or to spiders (Araneae) (Weygoldt and Paulus, 1979; Wheeler and Hayashi, 1998). The order is currently divided into 5 families, the monotypic Paracharontidae, which is regarded as sister lineage to all other recent species, the Charinidae (90+ species), the Charontidae (13 species), the Phrynichidae (35 species), and the Phrynidae (69 species) (M. Seiter, unpub.). The phylogenetic relationships among genera have been proposed by Weygoldt (1996) on the basis of morphological characters. This, however, remains the only comprehensive phylogenetic study on this group, and has a strong need of re-evaluation taking both morphological and molecular data into account. Further, the interspecific relations within genera remain highly unknown. Assessing new sets of characters may help

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http://dx.doi.org/10.1016/j.asd.2016.10.010
1467-8039/

in such future studies. Hence, one main goal of this study was to reveal the interspecific variation of the secretion crust (cerotegument) in whip-spiders.

Functionally, cerotegument structures are assumed to permit plastron respiration due to a water-repellent effect and the enclosure of air bubbles, as discussed for mites and centipedes (Krantz and Baker, 1982; Pugh et al., 1987; Adis et al., 1998; Raspotnig and Matischek, 2010). It is known that whip-spiders can survive a certain period submerged, which may ensure their survival during microhabitat flooding (Hebets and Chapman, 2000). Here we tested if the cerotegument-covered cuticle of whip-spiders has water repellent properties.

2. Material and methods

2.1. Study animals

The following material was investigated. **Abbreviations**: Collections: JW – collection of J.O. Wolff; MS – livestock and collection of M. Seiter (collection data of original animals given); SH – livestock of S. Huber (collection data of original animals given); SMF – Senckenberg Museum of Natural History Frankfurt, arachnological collection. Material used (see methods below for details): alc. – specimens preserved in 70% ethanol (whole animals or parts); cryo. – flash frozen samples of fresh (living) material; exu. – exuviae (freshly taken and air dried); liv. – living animals for wetting tests. Numbers in brackets indicate the number of individuals that were analysed.

Charinidae: *Charinus acosta* (Quintero 1983), Artemisa-Cuba, leg. M. Seiter, 2014, MS, exu. (1), liv. (4); *Charinus neocaledonicus* Simon 1895, Grand Terre-New Caledonia, SMF, alc. (1); *Sarax brachydactylus* Simon 1892, Luzon-Philippines, leg. M. Seiter 2014, MS, cryo. (1); *Sarax curioi* Giupponi & Miranda 2012, Panay-Philippines, leg. P. Scholwin 2014, MS, exu. (1). **Charontidae**: *Charon* cf. *grayi* (Gervais 1842), different populations (unclear species status, see (Weygoldt, 2002)): Luzon-Philippines, leg. M. Seiter 2014, MS, alc. (1); Cebu-Philippines, leg. M. Seiter 2014, MS, alc. (1); Negros-Philippines, leg. A. Jeffebeck 2014, MS, exu. (3), alc. (1), cryo. (1), liv. (11); *Stygophrynus* sp. (new species, description by M. Seiter, M. Schramm and J.O. Wolff, submitted for publication), Sulawesi-Indonesia, leg. P. Grabowitz 2014, MS, exu. (1). **Phrynidae**: *Heterophrynus* sp., Meta-Colombia, leg. M. Seiter 2014, MS, exu. (1), liv. (6); *Acanthophrynus coronatus* (Butler 1873), Mexico, leg. M. Gamache 2014, MS, exu. (1), liv. (11); *Paraphrynus carolynae* Armas 2012, Arizona–USA, leg. M. Gamache 2014, MS, exu. (1), liv. (4); *Paraphrynus cubensis* Quintero 1983, Artemisa-Cuba, leg. M. Seiter 2013, MS, exu. (1), liv. (8); *Paraphrynus robustus* (Franganillo 1931), Guantánamo-Cuba, leg. M. Seiter 2013, MS, exu. (1), liv. (6); *Paraphrynus viridiceps* (Pocock 1894), Cuba, leg. M. Seiter 2013, MS, exu. (1), liv. (7); *Paraphrynus* sp. (undescribed species, description in prep.), Morelos-Mexico, leg. J. Krall 2013, MS, exu. (1), liv. (12); *Phrynus barbadensis* (Pocock 1894), Barbados, leg. H. W. Auer 2014, MS, liv. (7); *Phrynus* aff. *barbadensis* (resembles *P. barbadensis*, but population located far away from type locality on Barbados), Aruba, leg. B. Giese 2011, MS, exu. (1), liv. (5); *Phrynus damonidaensis* Quintero 1981, Guantánamo-Cuba, leg. M. Seiter 2013, MS, exu. (2), liv. (4); *Phrynus decoratus* Teruel & Armas 2005, Cienfuegos-Cuba, leg. M. Seiter 2013, MS, exu. (1), liv. (3); *Phrynus exsul* Harvey 2002, Flores-Indonesien, leg. M. Seiter 2013, MS, exu. (2), liv. (7); *Phrynus goesii* Thorell 1889, St.Maartin, leg. M. Seiter 2014, MS, exu. (1); *Phrynus hispaniolae* Armas & Gonzáles 2001, Guantánamo-Cuba, leg. M. Seiter 2013, MS, liv. (4); *Phrynus longipes* (Pocock 1894), Peninsula Samana-Dominican Republik, leg. P. Grabowitz 2014, MS, exu. (3), cryo. (1), liv. (4); *Phrynus marginemaculatus* c. l. koch 1840, Puerto Plata, Dominican Republic, leg. M. Seiter 2015, MS, exu. (1); *Phrynus parvulus* Pocock 1902, Cayo-Belize, leg. M. Gamache 2014, MS, exu (1); *Phrynus pinarensis* Franganillo 1930, Pinar del Río-Cuba, leg. M. Seiter 2013, MS, exu. (1), liv. (3). **Phrynichidae**: *Damon annulatipes* (Wood 1869), Durban-South Africa, leg. P. Pumberger 2013, MS, exu. (1), liv. (7); *Damon medius* (Herbst 1797), Gambia, leg. M. Seiter 2011, exu. (1), liv. (10); *Euphrynichus bacillifer* (Gerstaecker 1873), Tanzania/Kenya (unknown exact locality, pet trade), leg. 2014 (unknown collector), exu. (3), liv. (15); *Musicodamon atlanteus*, Tata-Morocco, leg. S. Huber 2015, SH, exu. (1); *Phrynichus ceylonicus* (C. L. Koch 1843), Beliluhoya-Sri Lanka, leg. M. Seiter 2010, MS, SH, exu. (3); *Phrynichus jayakari* Pocock 1894, Salalah-Oman, leg. I. Hess 2013, MS, exu. (2); *Phrynichus deflersi arabicus* Weygoldt 2003, Oman, leg. S. Huber, SH, exu. (1). **Thelyphonidae** (as an outgroup, for comparison): *Typopeltis crucifer* Pocock 1894, Kenting-Taiwan, leg. J. Wolff 2013, JW, exu. (1), alc. (1).

Livestock animals were wild caught or bred from wild caught animals, and kept in plastic terraria with a 2 cm thick layer of humid turf-sand soil and pieces of bark as hiding places. Temperature was kept constant at 26–27 °C and relative humidity varied between 65 and 75%. Animals were fed every 7 days with cricket nymphs (*Acheta domesticus*).

2.2. Microscopy

For microscopy studies pieces of freshly collected, air dried exuviae (carapace and femur leg IV) were used. In some cases material preserved in ethanol was used (see material list above), and/or cryo-samples were taken for comparison (see methodology below). Furthermore a freshly ablated, air dried leg of a *Phrynus longipes* 6 h after moulting, and pieces of carapace and walking legs of the same individual deep frozen 24 h after moulting, were studied.

2.2.1. Light microscopy

For light microscopical images a multifocus stereo microscope (Leica M205 A, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a camera (Leica DFC420) was used.

2.2.2. Scanning electron microscopy

For scanning electron microscopy air dried samples were mounted on stubs with a double-sided carbon-rich adhesive tape, sputter coated with 10 nm Au–Pd and viewed in a Hitachi S-4800 SEM (Hitachi Ltd., Tokyo, Japan) at an acceleration voltage of 3.0 kV. Material stored in 70% ethanol was previously dehydrated in a series of increasing ethanol concentrations (80%, 90%, 100% and 100% on a molecular sieve), followed by critical point drying. In single species we studied the cerategumental structure in the 'living' state by shock freezing freshly ablated legs in liquid nitrogen. Such cryo samples were directly sputtered with 10 nm Au–Pd using the Gatan ALTO-2500 cryo system (Gatan Inc., Abingdon, UK) and viewed in the SEM with the stage cooled down to −120 °C.

2.3. Wetting tests

There is no standard method to determine the wettability of biological surfaces. There are different definitions of wettability, depending on what is measured (advancing vs. receding contact angle, local vs. global contact angle, roll-off angle, drop-shape). In micro-structured surfaces the local contact angle may highly differ from the global contact angle, and the latter therefore strongly depends on the size of the droplet. The effect of super-hydrophobicity only exists on the macro-level. Locally micro- or nano-structures are always wetted by the water. In order to measure the contact angle on the macro-scale, we did pre-tests on exuviae of

Please cite this article in press as: Wolff, J.O., et al., The water-repellent cerotegument of whip-spiders (Arachnida: Amblypygi), Arthropod Structure & Development (2016), http://dx.doi.org/10.1016/j.asd.2016.10.010