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OBSERVATION ON SILK PRODUCTION AND MORPHOLOGY OF SILK IN WATER MITES (ACARIFORMES: HYDRACHNIDIA)

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ABSTRACT: Adults of the following water mite species — *Piona coccinea* (C.L. Koch, 1836), *Limnesia undulata* (O.F. Müller, 1776), *Limnesia maculata* (O.F. Müller, 1776), *Limnesia undulatoides* (Davids, 1997), *Hydryphantes ruber* (de Geer, 1778) and *Mideopsis orbicularis* (O.F. Müller, 1776) maintaining in the laboratory for several months were shown to permanently produce various amount of silk in the form of long thin unbrunched threads. Morphology of these threads are similar in all studied species — they are stright, rigid, mostly hollow tubes of two dimension categories: thin 730±130 nm, and thick 1–2.5 μm in diameter. Predominance of different thread types varies freely in different mite species. Specific staining reveals neither DNA nor microbial walls in threads composition, so the microbial origin of threads is excluded. Staining with Calcofluor White M2R fluorochrome definitely indicates that these threads belong to arthropod silk. Organization of the threads is found to be simplest among known spiders and insect's silks. The observed silk formation does not correspond to the mite reproduction activity because has lasted from late summer till mid autumn where mites have already completed producing both eggs and spermatophores. Possible functions of the silk are discussed.

KEY WORDS: Parasitengona; adult water mites; spinning ability, silk threads

INTRODUCTION

The ability of silk production is one of the most remarkable functions of arthropods, in particular arachnids (Foelix 1996; Craig 1997, 2003) and insects (Kristensen 2003) and is thought to have evolved independently in different groups of Arthropoda (Vollrath et al. 1996; Craig 2003). From the general point of view, "silks are secreted fibrous materials that are deposited or spun by organisms" (Craig 2003, Preface xi). Biochemically, silks are protein threads composed of "fibrous proteins made up of repetitive sequences of amino acids" (Craig 2003, p. 3).

Silk production (spinning ability) among Acariformes is known for several families, such as Tetranychidae, Eriophyidae, Camerobiidae, Cunaxidae, Bdellidae (Wallace and Mahon 1972; Alberti and Ehrnsberger 1977; Bolland 1983; Gerson 1985; Manson and Gerson 1996; Alberti and Coons 1999). Silk in these mites provides an extremely wide spectrum of possible functions: producing of protective nests for eggs and other instars against predators, formation of guiding threads in the case of indirect sperm transfer for facilitation of revealing of spermatophores, hunting functions for capturing prey, dispersal of silk balls by wind or animal transport, cleaning of the nest space and eggs, as well as aggregation and communication (Schaller 1971; Witte 1991; Alberti and Coons 1999; Clotuche et al. 2011; Kanazawa et al. 2011; Le Goff et al. 2011; Fernandez et al. 2012; Yano 2012; etc.).

Specialized spinning organs — modified prosomal glands opening on the palp tips — are known involved in production of silk threads in tetranychid mites (Alberti and Coons 1999). In other acariform groups, organs producing silk are not still known with certainty. However, it is generally assumed that mites together with pseudoscorpions represent the phylogenetic lineage, in which webbing is mostly provided by the modified salivary glands (Gerson 1985).

In water mites (phalanx Hydrachnidia), extremely large and diverse group of the secondarily water arachnids, production of particular threads is shown associated with mating behavior in several families having indirect sperm transfer (Proctor 1992; Witte 1991; Alberti and Coons 1999; Witte and Döring 1999). In this behavioral pattern, males produce the type of guiding threads for spermatophores. Apart from this function, there are still no any evidences of silk production by water mites.

Initially, starting keeping of water mites in the laboratory, we pursued the purpose to receive eggs and larvae of the first laboratory generation. For this purpose, mites of different species were placed separately in the glass containers with pure water (see below). During experiments, we have surprisingly revealed that some time after completion of

the egg deposition, mites (females) have found themselves in the clouds of tiny whitish suspension. The period for which water become contained extraneous substance is rather short occupying few hours if not minutes. Generally, such situation lasted for months until late autumn when mites were died or taken for further experiments. Examination of these clouds with a light microscope has shown that they are composed of nearly indiscernible extremely thin and long transparent threads. Being leaved in the vial with a mite, these threads were sometimes interlaced into whitish flocks/clots supposedly due to the non-specific mite activity. This process was found to be most intensive in *L. undulata* and *H. ruber*. Mites moved within these whitish clouds without any obvious damages. It should be specially noted that before and during egg deposition, the production of threads wasn't identified with certainty, i.e. did not pay our attention. It should be also mentioned that flocks preserved in water did not undergo any changes or fouling with extraneous bacteria or fungi that may indicate the origin of these threads from mites exclusively.

To investigate this phenomenon specially, we continue keeping the mites separately and observed them for a long time trying to exclude any incidental influences and additional contamination and pollution of water containers. The nearest aim of the undertaken work was to determine the volume of the process and to give a detailed morphological description of threads. The fact that nobody has revealed earlier the water mite ability to produce silk unrelated to the mite's reproduction is unclear. It should be noted, however, that typically a comparatively low intensity of threads production can obviously be observed in most water mite species. This process can be surely traced only after searching of mites for a long period of time in a small water volume with a subsequent thorough microscopical analysis of water samples. Because almost all observations and experiments concerning silk were made on terrestrial mites, mostly tetranychids (Clotuche et al. 2011; Kanazawa et al. 2011; Le Goff et al. 2011; Fernandez et al. 2012; Yano 2012; etc.), no evident comparable biological/ecological models could be still proposed for analysis of silk production in water mites especially regarding their natural environment. Recently, a fluorescent dye, Calcofluor White M2R fluorochrome, was found appropriate for specific staining and revealing of arthropod silk (Johnson et al. 2006), including mites (Clotuche et al. 2009), that gives a reliable instrument for determination and visualization of various arthropod silks.

Taking into consideration the above mentioned arguments, the main purpose of this work is to demonstrate the obvious possibility of water mites to produce the type of silk in the laboratory condition and to provide detailed morphological characteristics of silk threads.

MATERIAL AND METHODS

Mites

All captured mites used in this study were females and were collected in the summer-autumn period 2012–2013 from the following localities of the European Russia:

— Smolensk Province, Demidovskiy District, National Park "Smolenskoye Poozerye," near Przhevalskoye town: temporary ponds in the outskirts of the 'Chistik' Teaching Centre (Smolensk State University) (55°30.22′N; 31°46.87′E, altitude 213 m), July 2012 — *Piona coccinea* (C.L. Koch, 1836), *Limnesia maculata* (O.F. Müller, 1776);

— Smolensk, lake Krivoye, former riverbed of Dnepr (dead channel) (54°46.55′N; 31°54.44′E, altitude 213 m), September, 2012 — *Limnesia undulata* (O.F. Müller, 1776);

— Smolensk Province, Demidovskiy district, National Park "Smolenskoye Poozerye," town Przhevalskoye, channel Sapscho-Svjatec (55°29.59′N; 31°49.06′E, altitude 154 m), September 2012 — *Hydryphantes ruber* (de Geer, 1778);

— Smolensk Province, Demidovskiy district, National Park "Smolenskoye Poozerye," near Przhevalskoye town, a pool at Kirovka village, lake Glubokoye (55°30.82′N; 31°47.84′E, altitude 160 m) — *Hydryphantes ruber* (de Geer, 1778), September 2012, *Limnesia undulatoides* (Davids, 1997), July 2013;

— Smolensk Province, Demidovskiy district, National Park "Smolenskoye Poozerye," near Przhevalskoye town, lake Chistik (55°30.23′N; 31°47.48′E, altitude 145 m), July 2013 — *Limnesia maculata* (O.F. Müller, 1776), *Mideopsis orbicularis* (O.F. Müller, 1776).

Laboratory observations

Two to five mites of each species were used in the experiment from the beginning. Mites were kept separately in glass containers 5–7 cm in diameter and 6–7 cm high with 3–4 cm water column at room temperature without feeding. For the

whole time of the experiment, we used pure bottled artesian water (certification of conformity N POCC RU.AE05.H02957, www.smolvoda.ru) distributed in Smolensk city. Each container was aerated for 3–5 hour daily with the help of a compressor. Approximately once a week, we washed containers and changed the water to reduce it from occasional fouling by fungi, algae and bacteria. A control container with fresh water and without mites was involved for comparison of the water condition. Also, a control container with a dead mite was used for observation and control on the typical fungal growth. We observed all containers once or several times a day. Generally, mites survived in the laboratory from the capturing up to winter, i.e. several months, being active for all this time, some mites occasionally died.

Light-microscope observations

Thread clots or separate threads were taken out from containers by small parts of the cover glasses placed on the bottom of containers or immediately by using microscopic needle for entire clot. Both temporary water and constant dry preparations were then made from the white silk flocks/clots of each mite species by mounting and stretching them as a thin one-row film on microscope slides. The specimens were then covered with a cover-glass and examined. Water preparations were examined immediately, dried up preparations were observed later using oil immersion. These preparations were examined and photographed with a Leica DM LS-2 light-optical microscope equipped with a Leica EC-3 digital camera (Laboratory of Parasitology, Zoological Institute of the Russian Academy of Science (ZIN RAS), St-Petersburg, Russia). The techniques of bright field (BF) and differential–interferential contrast (DIC) were performed with a Leica DM 5000-B microscope combined with a Leica DFC 320 camera (The Centre of Collective Use "TAXON", ZIN RAS, St-Petersburg, Russia) and with a Leica 2500 microscope equipped with a Leica DFC 500 camera (The Centre of Collective Use "CHROMAS" of St-Petersburg State University, St-Petersburg, Russia). Threads measurements were taken by a Leica TSP 5 morphometrical program in the center "CHROMAS".

Microbiological tests

Different methods of specific staining were applied for threads to exclude their microbial origin: (i) staining of heat fixed slides in aqueous gentian violet, the basic staining that interacts with bacterial (and fungal) cell wall and DNA; (ii) DNA-specific staining of vital and fixed material by 4',6-diamidino-2-phenylindole (DAPI), the results were appreciated in the St-Petersburg State university by using CSLM Leica TSP 5; (iii) standard Gram's stain protocol for differentiation of gram-positive and gram-negative bacteria (Department of Microbiology, St-Petersburg State University, St-Petersburg, Russia).

Fluorescent staining

A fluorescent stain (Calcofluor White M2R fluorochrome) was applied for water preserved clots and separate threads (natural condition) of *L. undulata* and *L. maculata* as alternative method for rapid detection of silk (Johnson et al. 2006; Clotuche et al. 2009) in accordance with the standard recommended protocol (http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Fluka/Datasheet/18909dat.pdf). Examination of the staining slides was performed in the Institute of Cytology of the Russian Academy of Science (CIN RAS, St-Petersburg, Russia) with (i) an inverted fluorescent microscope Axiovert 200M (Zeiss) combined with a digital camera Leica DFC420 under UV light using a FilterSet 02 and with (ii) CSLM Leica TCS SP5 using the smallest laser with an excitation wavelength 405 nm.

Transmission Electron Microscope (TEM) examination

For this purpose, a standard double fixation in glutaraldehyde and osmium tetroxide was applied for the entire thread clots of *L. undulata*. These clots were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2–7.4) for several days, washed in 0.2 M phosphate buffer for 1 hour, postfixed in 2% osmium tetroxide in 0.1 M phosphate buffer for 2 h, dehydrated in ethanol and acetone series, and finally embedded in an araldite mixture. Ultra-thin sections in transverse plane to a thread bunch were made on a Leica UC-6 ultramicrotome and, after staining with uranile-acetate and lead citrate, were examined with TEM Morgagni 268-D (digital visualization) at 80 kV (The Centre of Collective Use "TAXON", ZIN RAS, St-Petersburg, Russia).

Scanning Electron Microscope (SEM) examination

For SEM study, mites were initially fixed in 70% ethyl-alcohol, passed through increased alco-

hol series and then treated with hexamethyldisilazane (HMDS) for 5–10 min for providing natural shape and size of the mite body as alternative method to critical point drying. Immediately after these procedures specimens were covered with a platinum layer in an Eiko IB-5 apparatus, and then examined with SEM Quanta 250 at 10–20 kV.

Threads were mounted on cover glasses as a thin film, dried on air, covered with a platinum layer and examined with the same SEM. For ESEM mode, which provides an opportunity to observe wet samples, we used wet specimens (original silk threads of *L. undulata*) in a droplet of water. The samples were put on the peltier cooled stage in the vacuum chamber of the microscope and then pumping followed to the 700 Pa through two purging cycles (from 600–800 Pa), while purging cycles humidity in the vacuum chamber gains 100%. The specimen is still in a droplet of water. Slight decreasing of pressure makes the water vaporize, and the specimen became visible. For examination of silk threads the GSED detector at 20–30 kV in the SEM Quanta 250 was used (The Centre of Collective Use “TAXON”, ZIN RAS, St-Petersburg, Russia).

RESULTS

Laboratory observations

All examined mites (Fig. 1 A, E) produced white flocculent material (Fig. 1 B, C, F) consisting of extremely thin threads freely interlaced each other (Fig. 1 D). This process was seen most intensive in *L. undulata* (Fig. 1 B, C) and *H. ruber* (Fig. 1 E, F). Threads production occurred periodically for the whole time of observation from late summer till mid autumn with different intensity in different species, but generally occupied from several minutes to several hours each time. Mites produced threads while walking/running upon the bottom of the container (Fig. 1 B, E). The threads were stretched out from the posterior body portion that is most conspicuous in *L. undulata* (Fig. 1 B). This action is constantly accompanied by rapid movements of the fourth leg pair, which seems to ‘clean’ the body surface by jerky motions from forth to back (Fig. 1 A, E). A short time later, *L. undulata* may assemble the initial separate filaments into a type of clot (Fig. 1 C). In contrast, *L. maculata* and *L. undulatoides* produced few separate threads and never associated them into clots. *H. ruber* produced many filaments with the appearance of whitish flocks arranged further, with the help of the legs movements, into a freely organized white clot (Fig. 1 E, F).

In some studied species (*L. undulata, H. ruber*), the secretion was so intensive that a short time after changing of water (around several hours), a large amount of the non-organized flocculent material again appeared in the containers (Fig. 1 F). This phenomenon was also frequently observed in a drop of water with a mite placed on a microscope slide — in several minutes the water drop have become turbid from mite’s discharge. In other species this phenomenon was not so evident during visual observation and should be examined more carefully with a microscope. Fresh water in the control container always remains pure. No opalescence of water from the possible contamination of bacterial colonies was observed both in the working and in the control containers. Conversely, dead mite in a separate control container showed gradual fouling with a tight whitish cover of fungal mycelium that took around two-three weeks or even more. Mouthparts and especially palps do not obviously take part in the silk formation. Excretion by the studied water mites in the laboratory occurred rather rarely by ejection of whitish clouds of a fine granular material (guanine) through the excretory pore (anus) (for the excretory organ in the Parasitengona, see Shatrov 2010).

It should be noted that individual silk threads, especially in *L. undulata*, are not practically able to extension and, on the other hand, cannot be easily torn that is seen from the attempt to separate silk clot into smaller pieces.

Light-optical study

Light-microscopy of threads demonstrates that they are represented by extremely long colorless rigid hollow uniform tubes (Figs 2 A–D, 3 A, B) of two dimension categories: thin 730±130 nm, and thick 1–2.5 µm in diameter. Ratio of the different thread types varies in different mite species (Fig. 2 D), although thick threads are seen to predominate, as, for instance, in *H. ruber* and *L. undulata* (Fig. 2 A, C). In *M. orbicularis* thick and thin threads are equally represented (Fig. 2 B). The threads are never branched and may contain a core of the extracellular substance, especially in *L. undulata* (Figs 2 A, 3 B) (see also below). Constrictions of the thread’s walls of both the hollow threads and the threads containing a core were never observed. The threads are freely interlaced in the non-organized clots (Fig. 3 A), sometimes assembling into certain bundles.

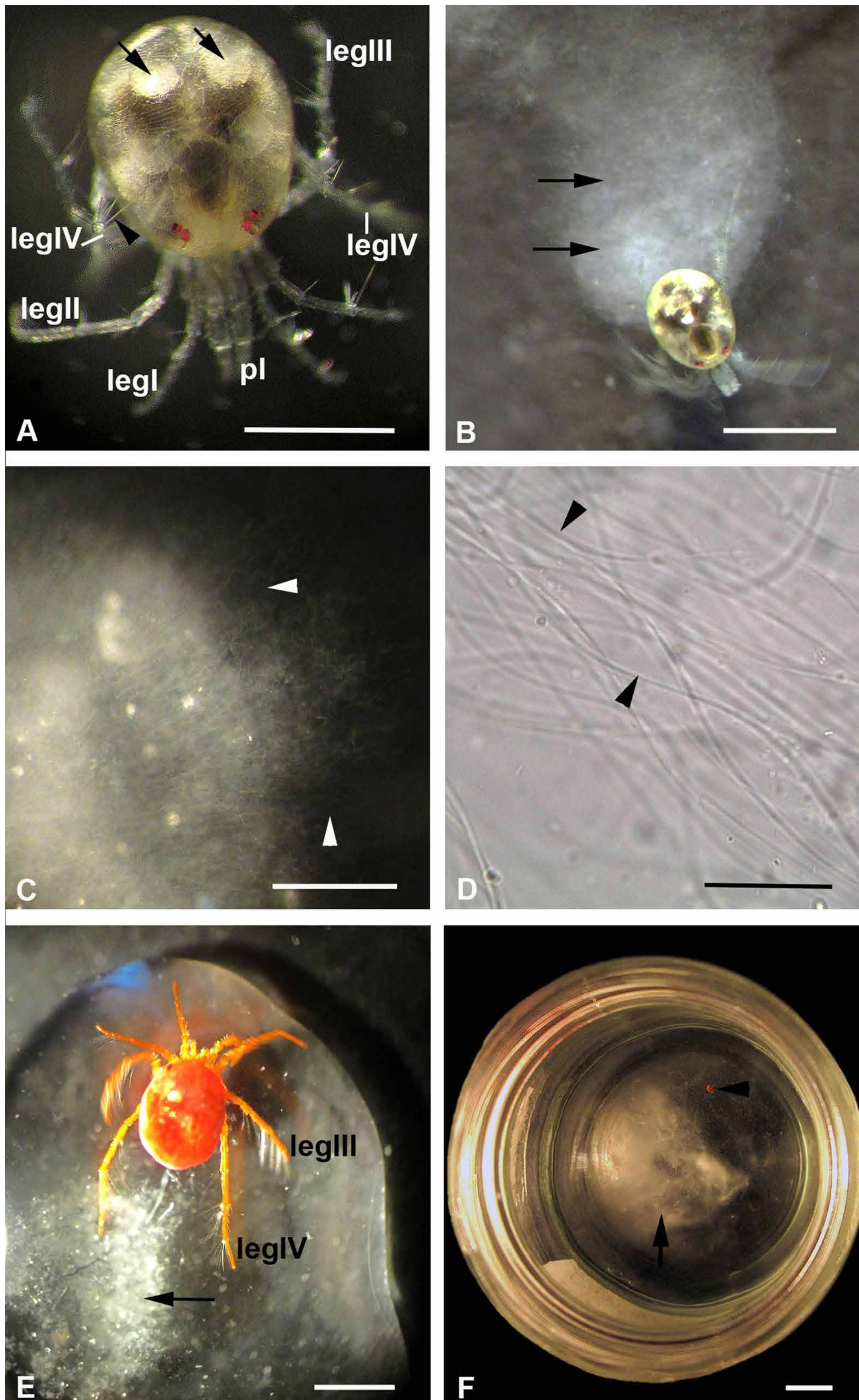

Fig. 1 (A–F). Mites and their silk in the laboratory observations. *L. undulata* (A–D), *H. ruber* (E–F). A — a mite combing the body surface with long hairs (*arrowhead*) on the legs IV. Note the dorsal dermal gland pairs (*arrows*), which are seen through the mite integument. Scale bar: 0.5 mm. B — the same mite producing much silk (*arrows*) that is detached from under the posterior body region. Scale bar: 1 mm. C — the portion of the silk clot with separate fine threads (*arrowheads*) seen on its periphery. Scale bar: 0.5 mm. D — freely interlaced threads (*arrowheads*) in a water film. Scale bar: 50 µm. E — the mite in a water drop pulling the tangle of silk (*arrow*). Scale bar: 1 mm. F — a glass container with water containing a single mite (*arrowhead*) and a newly produced silk in the form of white flocks (*arrow*). Scale bar: 10 mm.

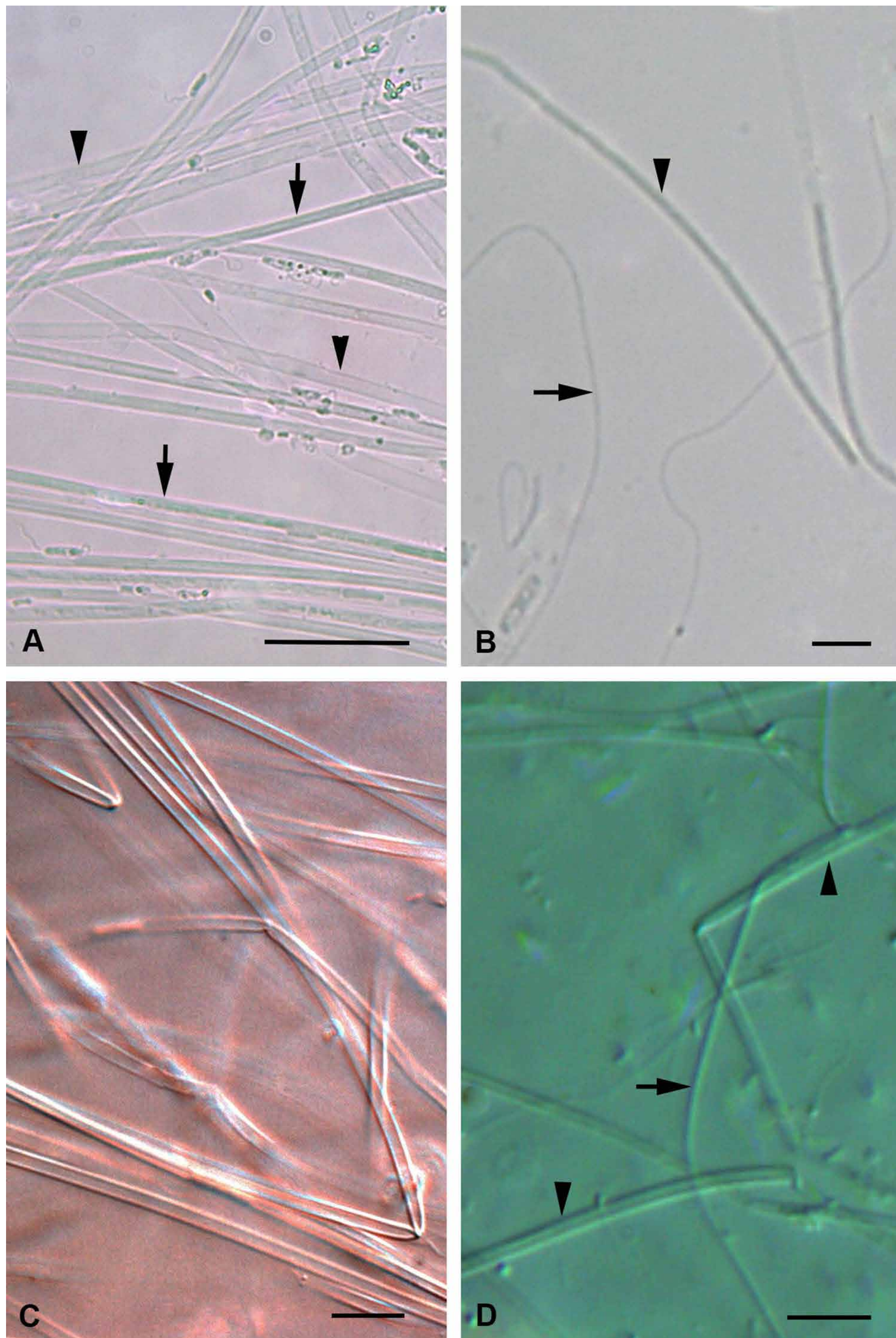

Fig. 2 (A–D). Silk threads on whole-mount light-optical unstained preparations under oil immersion. A — dried up preparation showing mostly hollow threads of *H. ruber* (*arrowheads*) freely plaited themselves. Arrows indicate threads with a core. Scale bar: 20 µm. B — dried up preparation showing thick (*arrow*) and thin (*arrowhead*) threads of *M. orbicularis*. Scale bar: 5 µm. C — DIC microscopy of *L. undulata* thick threads in water film. Scale bar: 10 µm. D — DIC microscopy of *L. maculata* thick (*arrowheads*) and thin (*arrow*) threads in water film. Scale bar: 10 µm.

Microbiological controls

Specific staining did not reveal DNA or microbial walls in threads composition. Staining of threads with gentian violet (Fig. 3 C) and with Gram's stain (Fig. 3 D) reveal the presence of accompanying both gram-positive and gram-negative bacteria in the samples (Fig. 3 D) but threads proper contain no bacteria or other DNA material. Characteristically that substance in the threads re-

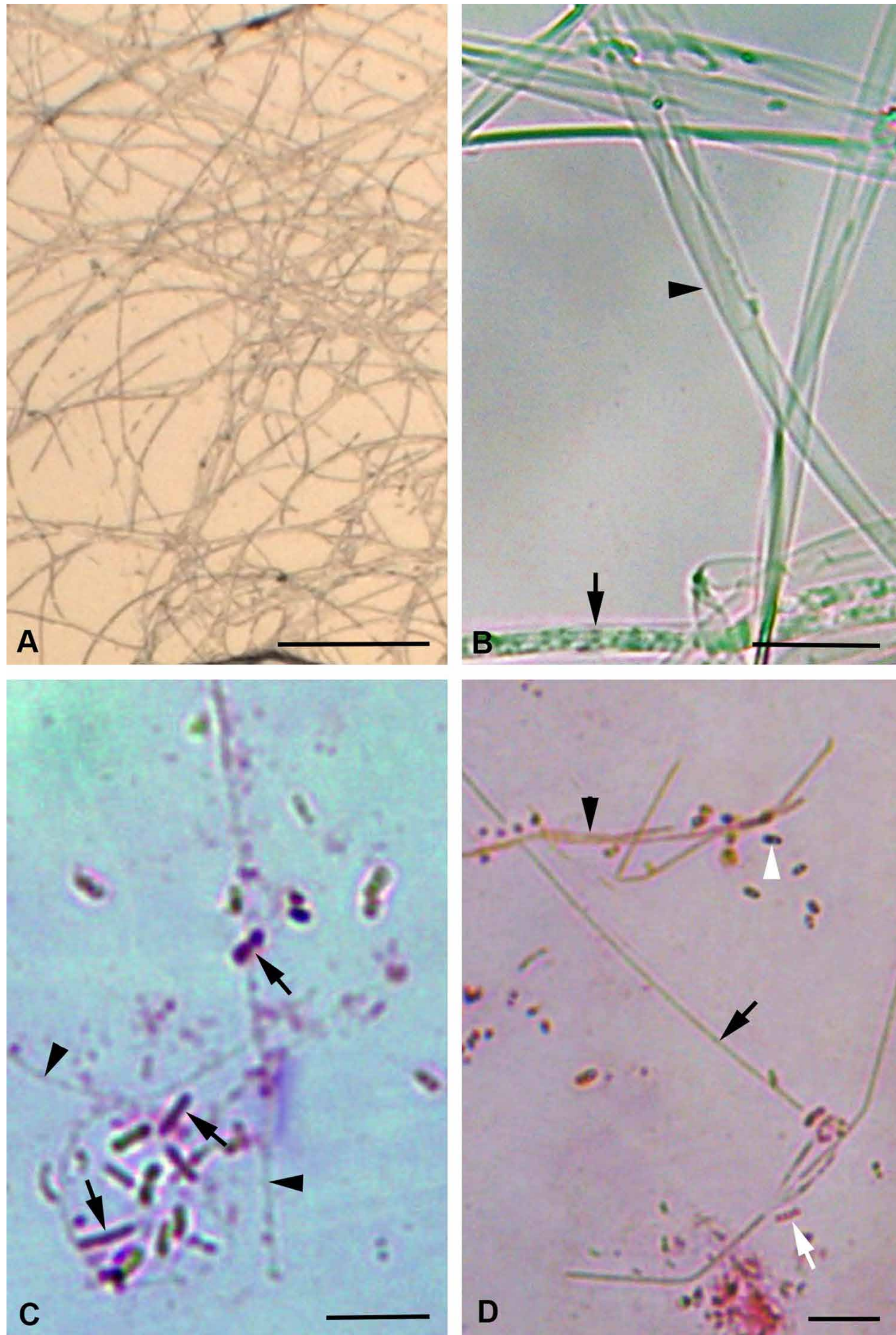

Fig. 3 (A–D). Light-microscopy of silk threads. A — general view of the silk clot of *L. undulata* mounted on microscope slide. Scale bar: 50 µm. B — freely interlaced dried up thick threads of *L. maculata* under oil immersion. Arrowhead indicates hollow thread, arrow points to thread with a core. Scale bar: 10 µm. C — gentian violet staining showing additional bacteria (*arrows*) and unstained thin threads (*arrowheads*) of *L. undulatoides*. Scale bar: 20 µm. D — gram stain showing gram-positive (*white arrowhead*) and gram-negative (*white arrow*) additional bacteria among unstained thick (*black arrowhead*) and thin (*black arrow*) threads of *L. maculata*. Note that thick threads are with a core. Scale bar: 10 µm.

mains unstained (Fig. 3 C, D) and so it cannot be classified as having cellular (bacterial) origin. Staining with fluorescent DNA specified stain (DAPI) shows that no characteristic fluorescence is seen related with threads. These tests have clearly indicated that threads related to water mite activity in the laboratory have no nucleic acids and so their bacterial origin can be excluded.

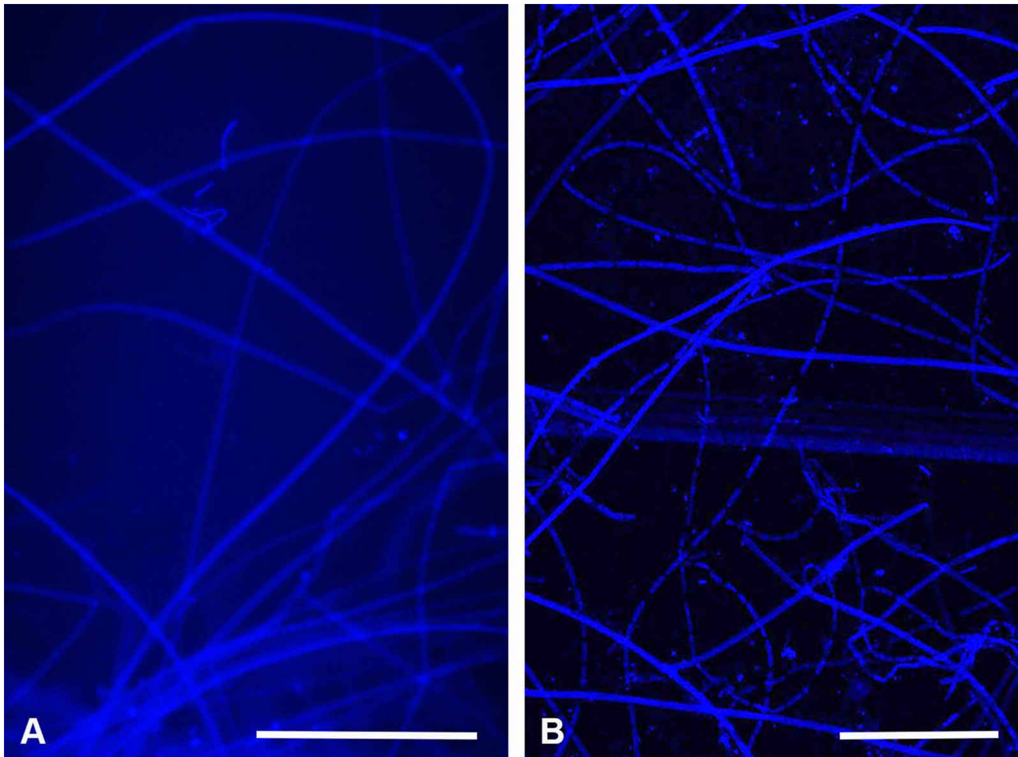

Fig. 4 (A–B). Fluorescent staining of silk threads of *L. maculata* (A) and *L. undulata* (B) with Calcofluor M2R fluorochrome. A — definitely stained silk threads in fluorescent microscope under UV with a FilterSet 02; B — silk threads in CLSM with 405 nm wavelength excitation. Scale bar: 50 μm in both figures.

Fluorescent control

Fluorescent staining with Calcofluor White M2R fluorochrome revealed distinct specific light-blue fluorescence for the whole length of threads (Fig. 4 A–B). The range of the wavelength emission in CLSM was found of 420 to 500 nm with the excitation laser of 405 nm wavelength. It is quite characteristically that the fluorescent examination did not reveal obvious accompanied microorganisms or fungi in the thread preparations. This finding has clearly indicated that threads discharging by water mites may be attributed as arthropod silk.

TEM study

TEM examination of thread bundle of *L. undulata* (Fig 5 A–D), mostly consisting of thick threads, showed that the threads lie separately and are represented by tube-like structures of round profile with the average diameter of about 1–2 μm (Fig. 5 A–C). The thread lumen is mostly totally free of contents or may contain a core of certain extracellular highly electron-dense coagulated substance mostly badly preserved during TEM preparation (Fig. 5 A, C). The thread walls significantly vary in their width from 50 to 200 nm and are composed of a micro-fibrillar material of moderate electron density without any obvious constant stratification. These fibrils are mostly arranged along the thread's axis (Fig. 5 D) so on transverse sections they look like fine granulation. No plasma membrane or any other cellular components are seen present in the thread's or their wall's composition. Sometimes, the wall's fibrils may split out themselves (Fig. 5 A), and the walls may show flat external and somewhat wavy internal layers. The detailed TEM organization of the thread's walls needs to be specially investigated.

SEM study

SEM revealed very long curved uniform threads chaotically crossing each other to perform a non-organized association (Fig. 6 A, B, D). 'Empty' threads mostly collapsed (Fig. 6 C), whereas threads with a core inside remained nearly 'natural' in shape (Fig. 6 C). Sometimes, residuals of the internal substance may be also seen in the collapsed threads (Fig. 6 C). The threads possess a smooth surface and are never branched but may be arranged in bundles consisting of several individual threads, as in *H. ruber* (Fig. 6 D) and *L. undulata*. In the latter species, the thread clots

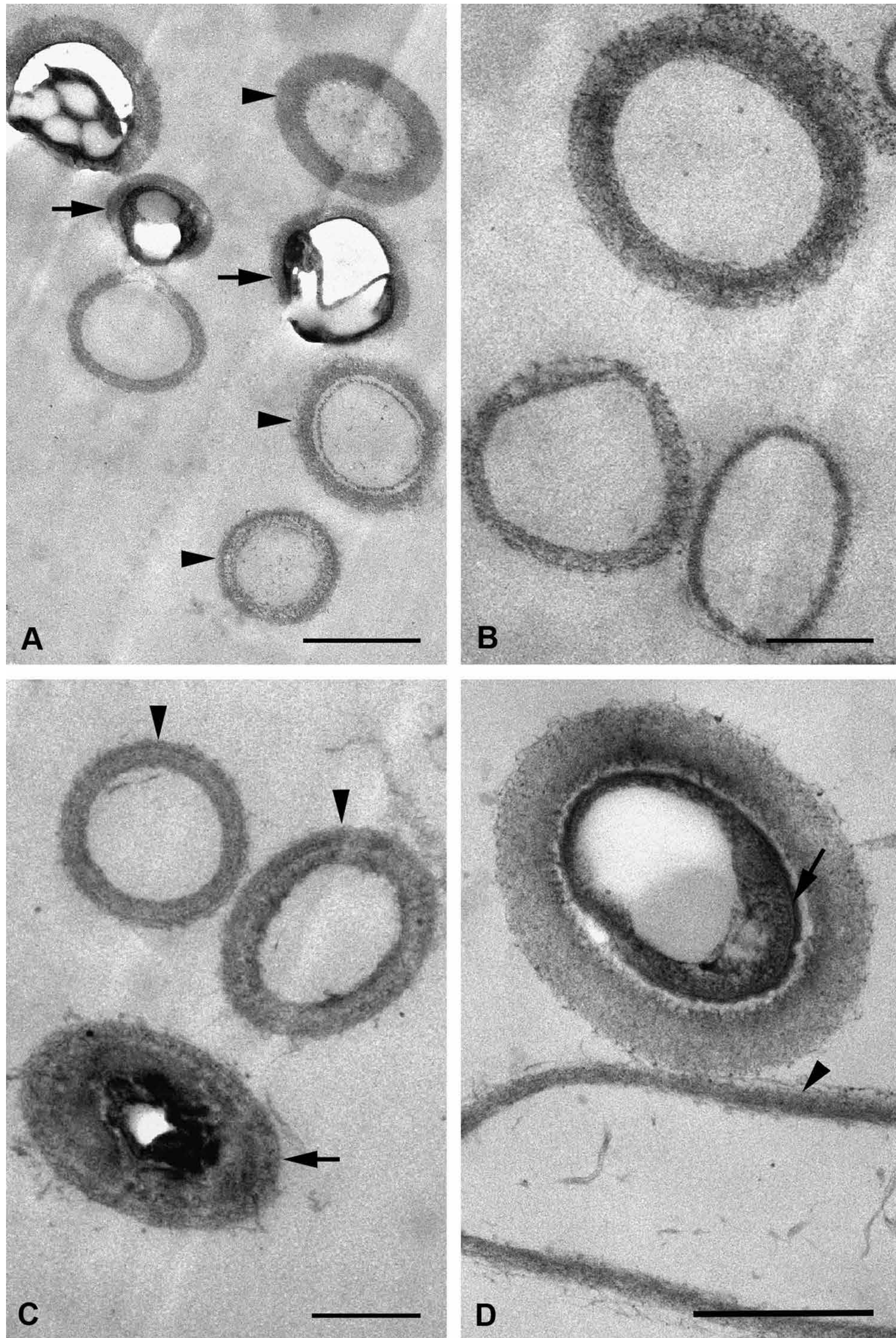

Fig. 5 (A–D). TEM of *L. undulata* silk threads. A — round profiles of hollow threads (*arrowheads*) and of threads containing an electron-dense vacuolated core (*arrows*), nearly equally presented, on transverse section. Note that the threads slightly vary both in their width and in thickness of their loosely structured walls. Scale bar: 1 µm. B — hollow threads with walls of different thickness. Note that there is no plasma membrane presented in the wall composition from inside. Scale bar: 0.5 µm. C — hollow threads (*arrowheads*) and thread with an electron-dense vacuolated core (*arrow*). Scale bar: 0.5 µm. D — transverse and longitudinal profiles of threads with walls of various thickness built of micro-fibrils axially arranged (*arrowhead*). Note that there is no plasma membrane in the wall composition. Note also that the partly destroyed electron-dense vacuolated extracellular core of the transverse sectioned thread is separated from the wall by a thin electron-dense layer (*arrow*). Scale bar: 0.5 µm.

mostly consist of thick threads, and thin threads occur extremely rarely (Fig. 6 B).

Examinations of the mites in SEM revealed small terminal openings of dermal glands (glandularia) scattered throughout the body surface (Fig. 7 A, C, F). No other foramens or conspicuous pore orifices were observed on the cuticle of the studied specimens. In contrast with the common opinion

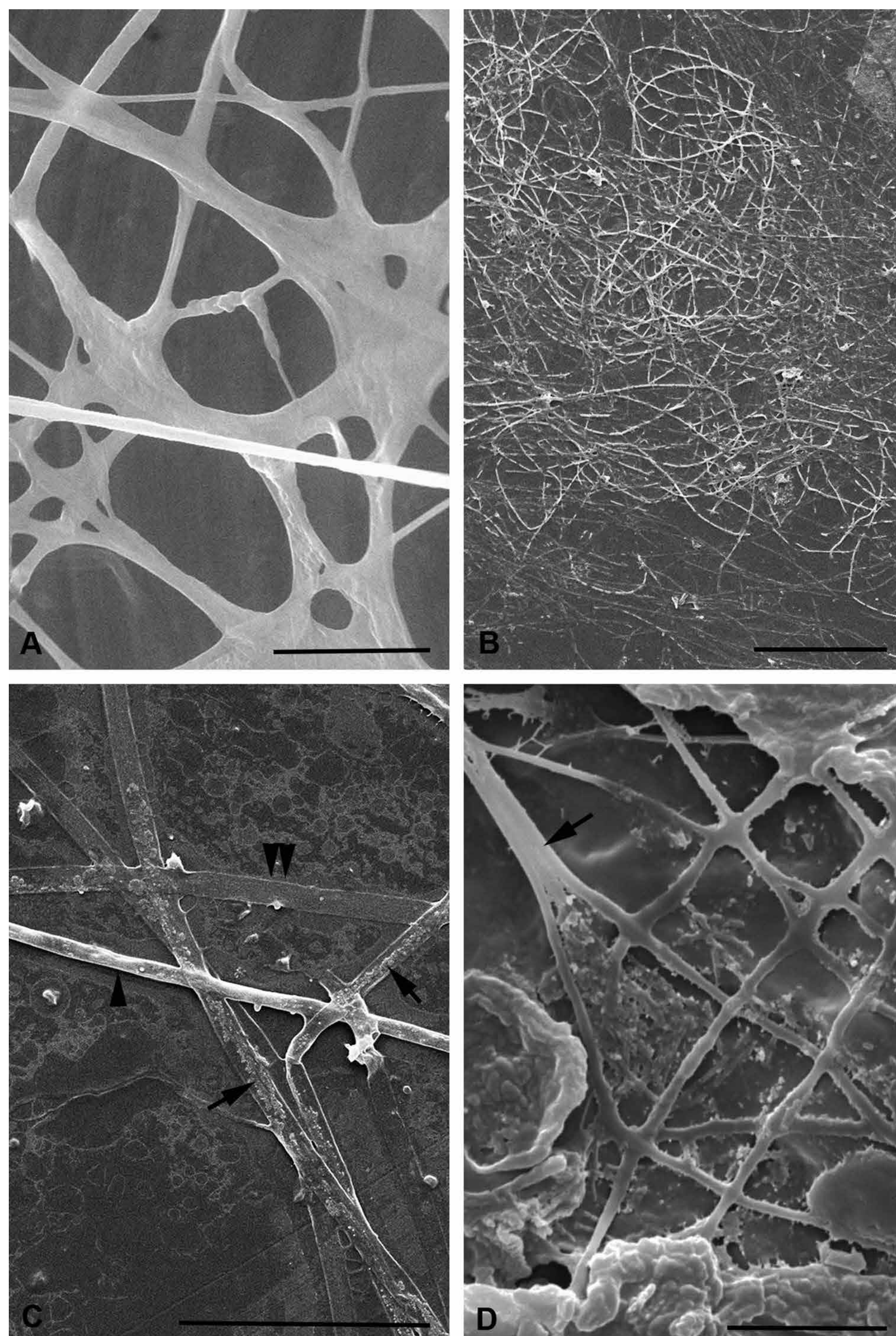

Fig. 6 (A–D). SEM of silk threads. *L. undulata* (A–C), *H. ruber* (D). A — ESEM showing portion of silk clot with freely interlaced threads. Scale bar: 10 μm. B — silk clot where hollow threads and threads with a core are seen equally proportioned. Scale bar: 100 μm. C — crossing threads both with a core (*arrowhead*) and hollow (*double arrowhead*). Note substance particles in thread replicas (*arrows*). Scale bar: 20 μm. D — crossing threads and particular thread bundle (*arrow*) partly contaminated with extraneous substance. Scale bar: 10 μm.

(Sokolov 1940), some abdominal glandularia, at least, those at the putative position of epimeroglandularia 4, ventroglandularia 2 and 3 in *L. undulata* (Fig. 7 B), epimeroglandularia 4 in *L. maculata* and epimeroglandularia 4 in *P. coccinea* (for the nomenclature of glandularia, see Wiles 1997) lack the accompanying setae. In *L. maculata*, some dorsal glandularia show secretion/extrusion coming out from the dermal gland orifices like relatively thick wavy bands (Fig. 7 C–D). There were observed no other direct or indirect evidences of the possible secretion of any substances through the mite cuticle.

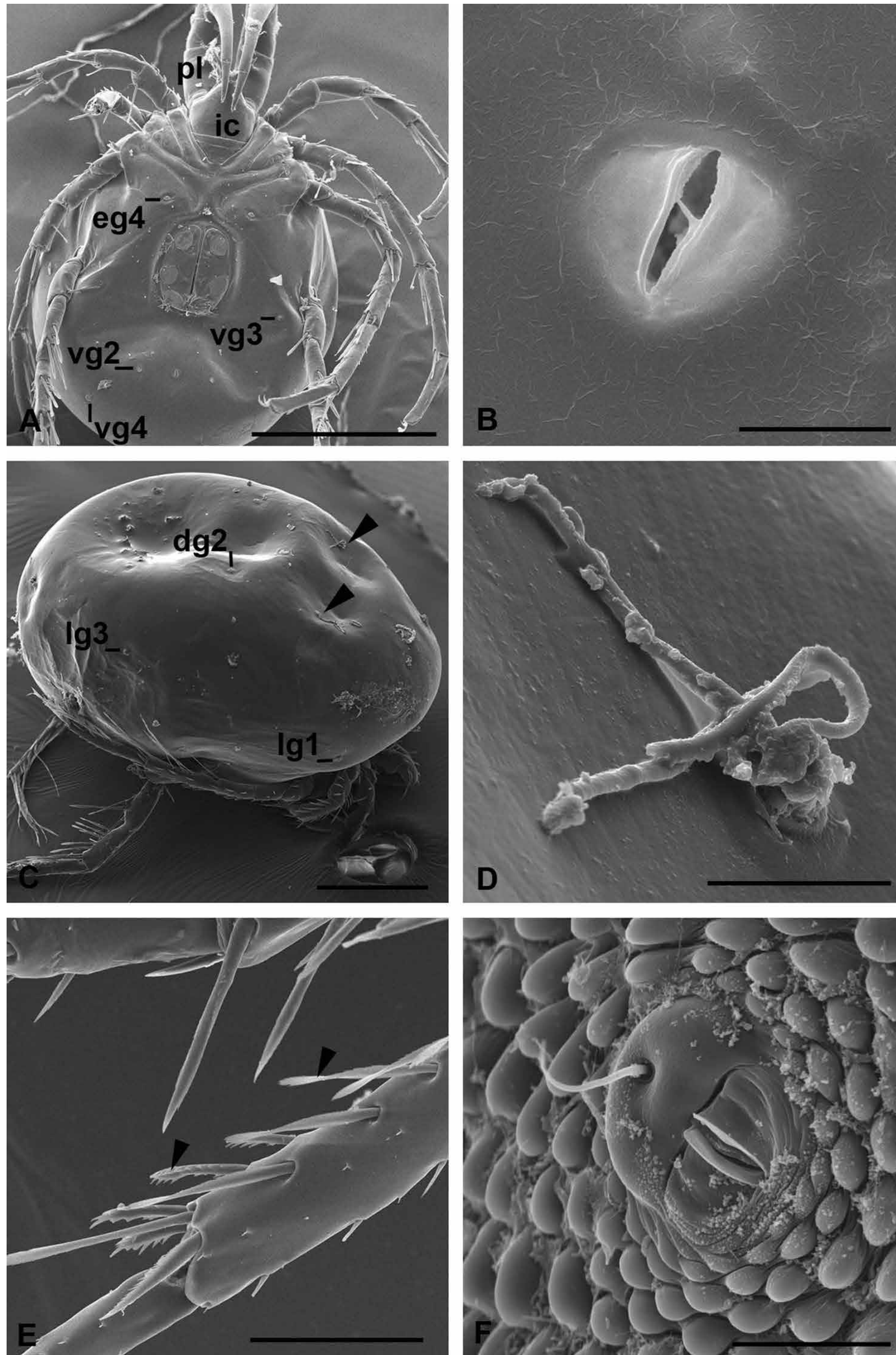

Fig. 7 (A–F). Details of the mite organization with regard to silk formation. SEM. *L. undulata* (A–B), *L. maculata* (C–E) and *H. ruber* (F). A — ventral body region showing some ventral glandularia (dermal gland openings). Scale bar: 500 µm. B — one of the ventroglandularia 2 without accompanied seta. Note flat body cuticle without pore orifices. Scale bar: 10 µm. C — lateral view of a mite showing some dorsal and lateral glandularia and extrusion from both of the dorsoglandularia 1 (*arrowheads*). Scale bar: 500 µm. D — secretion coming out from the dorsoglandularia 1. Scale bar: 40 µm. E — propeller screwed setae (*arrowheads*) with indented edges situated on the medial side of tibia III. Scale bar: 100 µm. F — ventroglandularia 4 with accompanied seta. Scale bar: 20 µm.

List of abbreviations on figures:
dg2 — dorsoglandularia 2; eg4 — epimeroglandularia 4; ic — infracapitulum; legI – legIV — legs of I to IV pairs; lg1, lg3 — lateroglandularia 1 and 3; pl — palp; vg2 – vg4 — ventroglandularia 2–4.

The mite's legs bear different kind of setae but flattened, twisted serrate-edged setae predominate on the legs III and IV, in particular in *L. maculata* (Fig. 7 E) and *L. undulata*. The edges of

these setae are armed with numerous small denticles (Fig. 7 E), which may act in possible combing the secretion.

DISCUSSION

This study inevitably shows that water mites produce extremely thin tube-like threads, and these threads may be attributed as silk. The latter is definitely proved by a specific fluorescent stain that is highly corresponded with the same stain applied for the spider mites' silk (Clotuche et al. 2009). The absence of plasma membrane and any other cell components in the threads' composition as well as the total lack of constrictions and branches in the thread organization obviously indicate that threads cannot be considered as fungal mycelium or bacterial associations. However, the chemical composition and exact functions of this water mite silk still remain unknown. Inability to extension and much strength, in comparison with spider silk (Denny 1976; Craig 1997, 2003), may show that fibrous proteins play a minimal role in the biochemistry of the water mite's silk, in particular, in the hollow threads. Conversely, it may be supposed that the water mite's silk threads are mostly composed of chitin micro-fibrils hard to extension. This also may be confirmed by the intensive stain of the mite threads with Calcofluor fluorochrome, highly specific to chitin. Taking into account that dermal glands (see below), which only may produce silk in water mites are of ectodermal origin (Shatrov 2013), and the main function of the ectoderm layer is formation of chitinous cuticle, this supposition is not out of sense.

In any case, it is clear that the observed silk production is not related to the mite's reproductive activity. To recognize this phenomenon in the given water mite species with certainty one must perform careful examination of a mite maintaining in the laboratory in a small volume of water for a long time preferably in late summer and autumn.

The feeding organs — the mouth parts and the prosomal salivary glands — do not apparently involve in this process. Conversely, it may be carefully supposed that dermal glands, evolutionary acquisition of this phyletic lineage, can take part in this process (Shatrov 2013). This supposition contradicts the present opinion that in the mite phyletic lineage only transformed salivary glands may be involved in the silk production (Gerson, 1985), whereas, as it is shown in other arachnid groups, in particular spiders, abdominal glands, independent of the digestive system, are involved in this function (Foelix 1996, Craig 2003) (see below).

In the mite species studied, the webbing does not apparently serve for spermatophore guidance, as it is observed in some water mite families (Proctor 1992; Witte 1991; Alberti and Coons 1999) because all examined specimens were females. At the same time, it was shown that the studied species may demonstrate various reproductive behaviors and show complete (*Limnesia*), incomplete (*Hydryphantes*) dissociation and even direct sperm transfer (*Piona*) (Proctor 1992). The possible sources of the guiding threads in the case of males are particular genital glands (glandular testes cells) (Witte and Döring 1999).

Such long and intensive thread secretion, as in our observations, cannot also be focused on egg protection or forming nest for the early developmental stages because larvae of the studied species were hatched out from the deposited eggs and become active long before webbing would stop late in autumn. I.I. Sokolov (Sokolov 1925; Sokolow 1977) studying water mite egg clutches, did not observe the developed egg nests. On the other hand, some individual water mites of unknown species captured during our collections in spring were enclosed within whitish fibrous cover. If it is true, one of the possible functions of the silk is protection of mites in winter period. It is shown that females of Camerobiidae and Tetranychidae deposit their eggs in or under the webs (Bolland 1983; Gerson 1985; Clotuche et al. 2011) and thus form a dense cover, nest or a canopy of silk above freshly laid eggs or above a whole mite colony, as it is also noted for some members of Eriophyidae (Manson and Gerson 1996). Moreover, in Tetranychidae larvae and nymphs are also shown to spin (Gerson 1985). Conversely, heteromorphic water mite larvae, for instance, larvae of *Piona carnea* (Koch, 1836), cannot produce threads because they are devoid of dermal glands and their podocephalic salivary glands, opening into the subcheliceral space (Shatrov 2012), serve, apparently, for extra-intestinal digestion of the host tissue. The palps of these larvae are also free of additional spinning organs.

As in the spider mites (Hazan et al. 1974; Clotuche et al. 2011; Le Goff et al. 2011; Fernandez et al. 2012; Yano 2012), thread production in water mites does not depend on feeding. In fact, all mites under experiment continued producing threads for months after they have been captured and, consequently, have stopped feeding. Water mites stud-

ied, as it was also observed in spider mites (Hazan et al. 1974, Gerson 1985), became immobile and did not produce silk in darkness (at night) and resumed it on the light. These examples show that in various mite groups having probably spinning organs of different evolution origin, general physiological stimuli for thread secretion appears to be similar.

It is shown that in tetranychids, the large unicellular glands located mostly within the gnathosoma and opening on the palp tips are responsible for threads production (Alberti and Storch 1974; Mothes and Seitz 1981; Alberti and Crooker 1985; Alberti and Coons 1999). Using special fluorescent staining, the threads of spider mites were shown to be silk (Clotuche et al. 2009). In the case of Bdellidae, Cunaxidae (Alberti and Ehrnsberger 1977), Camerobiidae (Bolland 1983) and Eriophyidae (Manson and Gerson 1996) the sources of webbing are not known with certainty but most probably these are glands related to modified prosomal glands and mouthparts (see for details Alberti and Coons 1999). By this character, water mites differ significantly from all other mite families studied so far because they do not use prosomal glands for this function. Most likely, they use for thread production large and usually well developed abdominal dermal glands (Shatrov 2008, 2013), but this supposition cannot still be proved definitely at the moment. Nevertheless, if it is true, water mites probably belong to the group where protein-secreting silk/dermal glands have evolved *de novo*, especially that all silk-producing cells are thought originated from an ectoderm cell lineage (Craig 2003). In accordance with this assumption, the revealed organization of the silk threads in water mites is simplest, especially in comparison, for instance, with those of spiders (Stubbs et al. 1992; Foelix 1996; Vollrath et al. 1996) and insects (Kebede et al. 2014). It is shown, in particular, that threads of the golden orb-weaver spider *Nephila madagascariensis* (Vinson, 1863) (Nephiliidae) dragline silk are composed of a micro-fibril wall with the outer coating and the inner membrane encompassing a milky core (Vollrath et al. 1996) that is much more complicated than in water mite's threads. Generally, although various spider silks are found organized biochemically and structurally difficult (Foelix 1996), no comprehensive and comparative works are still available on their ultrastructure. Nevertheless, it may be supposed that the wall of water mite threads is build up of chaotically organized fibroin and/or chitin fibers immersed into a homogeneous matrix, whereas a core, if present, remains to be gel-like or liquid as released from the mite. As it was shown in spiders, amino acids of silks are preserved in the silk gland as a liquid fraction and transformed into fibers during discharging from the animal by tension and spinning (Craig 2003). A similar process probably occurs in water mites, although secretion material in their dermal glands is found having an electron-dense protein nature (Shatrov 2008, 2013) in contrast with some spiders (Kovoor and Zylberberg 1980, 1982) and insects (Akai 1982) where the silk gland secretions are predominantly electron-clear. However, it is evident from the general consideration that secretion releasing into water must have a much greater density than that releasing on air, where it inevitably solidifies.

Among other Parasitengona, spinning ability is known in Erythraeidae and Trombidiidae (Witte 1984, 1991; Witte and Döring 1999; Alberti and Coons 1999), where various probably silk threads, seem to function as signal threads guiding to spermatophores or in spermatophore protection as well. In adult trombiculid mites (Trombiculidae), living deep in soil, webbing is not apparently shown (Shatrov 2000). In this group, spermatophores, laid by males on the substrate, are not provided with guiding threads (Shatrov 2000).

The biological role of the thread production in water mites is still unfortunately unclear. It should be noted that formation of silk in water is shown for the first time, and there are no reliable criteria for evaluation of this process. General similarity of the water mite silk in different mite species studied in the present work, both plesiomorph (*Hydryphantes*) and derived (*Mideopsis*), with contrast to spiders (Craig 2003), may indicate that water mites are not as diverse as it looks from their external morphology. On the other hand, a great variability of the dermal/silk gland morphology (A.B. Shatrov, unpublished) may point to the fact that either the water mite silk may be really different in structure and functions, or dermal glands may function in quite different ways.

In comparison with insect orders, only representatives of Embiidina demonstrate specialized silk glands of the 'dermal' origin. The latter function in protection, whereas initially silks are thought used for reproductive purposes and were produced by colleterial glands, and only later for protection and foraging (Craig 2003). By this analogy, it may be supposed that water mite's der-

mal glands were elaborated *de novo* apart from reproductive function and thus were specialized for protection by quite different pathways. If this silk is also used as a capture mechanism, as it is shown in Cunaxidae and Bdellidae (Alberti and Ehrnsberger 1977; Alberti and Coons 1999) is unclear. Generally, these water mite's threads are expected forming for protection of mites in unfavorable and, moreover, unusual external conditions. In any case, this characteristic brings water mites together with other arachnids (in particular, spiders) that exploit special abdominal glands for producing various nets.

It is generally assumed, however, that dermal glands serve for defense of water mites from potential predators (fishes), especially when dermal glands are accompanied with 'trigger' sensitive hair (sensillum) (Kerfoot 1982; Alberti and Coons 1999; Kirstein and Martin 2009, 2010; Shatrov 2013). This supposition does not strictly contradict with the proposed protective functions of the glands. The absence of sensillum in the vicinity of some glandularia in the species studied may imply particular specialization in the gland functions in the given species.

Possible mechanism of transformation of the dermal gland's secretion/extrusion into the threads remains unclear. From the mechanical point of view this process may be realized by the movements of the fourth leg pair along the body from forth to back that would comb and separate the dermal gland secretion into much thinner threads by the propeller-like setae on the distal leg segments, as it is processed in spiders (Foelix 1996). Extremely long capillary-like nanotubes are the obvious result of this action. Biological meanings of these threads/nanotubes in relation to the water mite biology and their physical properties are still waiting for their solutions.

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