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Confocal Microscopy and Molecular Analyses Reveal Anal Secretory Apparatus in Immatures and Recover Transcontinental Clade of Gall Mites (Eriophyoidea) from Tamarisks

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Abstract: Tamarisk is an invasive evergreen shrub native to arid regions of Africa and Eurasia and is considered a weed in some countries with dry climates. The complex of gall mites from tamarisks includes fourteen species from four genera of the family Eriophyidae. We reinvestigated the type species of the genus *Dicruvasates*, *D. tamaricis* from Egypt, described *D. ngubani* **n. sp.** from *Tamarix usneoides* from South Africa, and revised the generic concept of *Dicruvasates*. This genus possesses cuticular plates marking longitudinal opisthosomal ridges typical of various phyllocoptine genera, e.g., *Echinacrus*. We sequenced three genes of *Dicruvasates* and *Phyllocoptes* mites from *Tamarix* and *Echinacrus* from *Frangula*, combined our sequences with the data from GenBank, and performed single-gene phylogenetic analyses. All analyses inferred the following: (1) a transcontinental clade *Tam* comprising *Dicruvasates* + *Aceria* + *Phyllocoptes* from *Tamarix*; (2) *Echinacrus* distantly related to *Dicruvasates*, suggesting the homoplastic nature of the cuticular plates on the opisthosomal ridges, and (3) polyphyly of the genera *Aceria* and *Phyllocoptes*. We also investigated partially cleared specimens of *Dicruvasates* under CLSM and, for the first time, visualized a well-developed anal secretory apparatus (ASA) in the immatures of gall mites, which was previously shown to be a specialized system evolved in Eriophyoidea for silk production. We hypothesize that the ASA contributes to finding nymphs by conspecific males and advocate for the importance of the future molecular biology studies of gall mite silk for developing new methods for controlling mites based on the knowledge of their ecology, behavior, and silk gene genetics.

Keywords: acariform mites; *Tamarix*; *Dicruvasates*; arthropod structure; host-specificity; silk

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1. Introduction

Gall mites of the superfamily Eriophyoidea (Acariformes) are microscopic host-specific phytoparasites permanently associated with higher vascular plants. Although phylogenetic patterns in gall mites are generally uncorrelated with those in plants, molecular studies of the last decade showed that monophyletic groups of eriophyoids associated with supraspecific host plant taxa may have arisen during their evolution [1–6]. As a result of the feeding activities of gall mites, various pathological effects appeared in plants infested by eriophyoids, some of them seriously suppressing their viability [7]. Different studies

showed eriophyoid mites are appropriate agents for controlling weeds, which is the other but positive side of their harmfulness [8,9].

Tamarisk or saltceder (g. *Tamarix*, Tamaricaceae) is one of the weed plants that was proposed to be a target for biocontrol with the aid of gall mites [10–12]. The genus *Tamarix* comprises 57 valid species [13]. It is a multi-branched evergreen shrub widely distributed in temperate and subtropical regions in South Europe, Asia, and Africa [14]. In these regions, tamarisks are native, whereas only a few introduced species are known in North America and Australia [15]. In different countries with dry climates, tamarisks may be highly invasive, especially when introduced, due to their outstanding salt and water resistance and competition with local plants [11,16].

The complex of eriophyoid mites from *Tamarix* includes a relatively low number of species (13), all of them belong to four genera (*Aceria*, *Eriophyes*, *Dicruvasates*, and *Phyllocoptes*) of the family Eriophyidae [17,18]. Among them, only some *Aceria* spp. cause distinct damage to their hosts in the form of variously shaped galls. Other species were described as vagrants, which cause no visible damage to tamarisks or were associated with rust, although this association has never been experimentally tested and was estimated based only on the general appearance of the entire infested plant.

Among phyllocoptine mites from *Tamarix* (genera *Phyllocoptes* and *Dicruvasates*), the species *Phyllocoptes immigrans* Keifer, 1940, has an unusually wide distribution, which putatively reflects the wide host specificity of the mite and the introductions of its host (Figure 1). The other species of this genus from tamarisk, *Phyllocoptes bilobospinosus* Chetverikov, 2018, is remarkable in that it was the first phyllocoptine species with documented elements of the anal secretory apparatus (ASA), which was revealed in partially cleared females [19]. Later, the presence of the ASA was demonstrated in different unrelated eriophyoid taxa and investigated in detail in *Aberoptus* Keifer, 1951, a genus of silk-producing mites living under web mats on the leaves of their hosts [20]. All findings of the ASA concern adult mites. It is unknown whether immatures lack ASA or if they have it at least in rudimentary form.

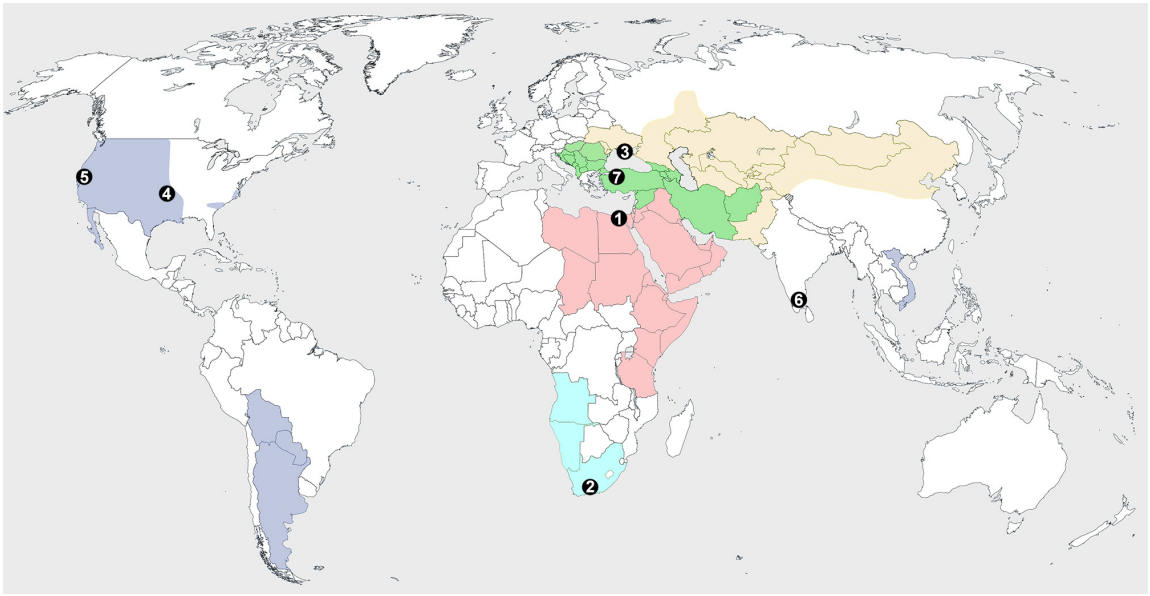


Figure 1. Findings of *Dicruvasates* (1–2) and *Phyllocoptes* (3–7) mites on *Tamarix* spp. 1—type locality of *D. tamaricis* from *T. nilotica* in Egypt, 2—type locality of *D. ngubani* **n. sp.** from *T. usneoides* in South Africa, 3 and 4—type (3) and additional (4) localities of *P. bilobospinosus* from *T. tetrandra* in Crimea (3) and from *T. ramosissima* in USA (4), 5—type locality of *P. immigrans* from *T. ramosissima* in USA, 6 and 7—findings of *P. immigrans* on *Tamarix chinensis* Lour. (=*T. plumosa* Carrière) in India (6) and on *T. parviflora* in Turkey (7). Natural distributions of four *Tamarix* spp. are approximated based on [21] and indicated by color: light blue—*T. usneoides*, pink—*T. nilotica*, green—*T. tetrandra*, orange—*T. ramosissima*, and pale violet—introduction of *T. ramosissima*. Illustration—P.E.Chetverikov.

In 1995, the monotypic genus *Dicruvasates* Abou-Awad and El-Borolossy was described based on material from the native African tamarisk *T. nilotica* from Egypt. According to the original description of the type species *D. tamaricis* [22], it has unique paired horn-shaped processes of unknown function and origin, protruding above cheliceral bases. Additionally, it has distinct longitudinal opisthosomal ridges marked with small cuticular plates, the state often observed in different phyllocoptine genera, e.g., in some *Abacarus* Keifer, 1944, *Echinacrus* Keifer, 1966, or *Quintalitus* Meyer, 1989 [23,24]. The genus *Echinacrus*, with five rows of triangular opisthosomal plates, includes five species described from the USA, Finland, Thailand, Egypt, and South Africa from three host-plant families (Rhamnaceae, Fabaceae, and Meliaceae). Remarkably, two of them, *E. acaciae* (Smith-Meyer, 1989) and *E. halawanii* Elhalawany, 2013, were described from the same African countries where recent taxonomic studies on eriophyoids from tamarisks were carried out [18,25].

During our field surveys in South Africa and Egypt, we collected eriophyoid mites from indigenous African tamarisks targeting rarely encountered members of *Dicruvasates*. We recollected *D. tamaricis* near its type locality in Egypt and found a new species of *Dicruvasates* on the endemic southern African tamarisk *T. usneoides*. In this paper, we aimed to do the following: (a) Describe the new species, provide a supplementary description of *D. tamaricis*, and revise the generic concept of *Dicruvasates*; (b) Estimate the phylogenetic position of *Dicruvasates* including a possible relationship with *Echinacrus*; (c) Test the monophyly of the complex of *Tamarix*-associated eriophyid species; and (d) Test the hypothesis that the ASA is present in all instars of gall mites using *Dicruvasates* mites as an example.

2. Materials and Methods

Collection and morphological measurements. The leaves and branches of tamarisks and *Frangula alnus* were sampled in South Africa, Egypt, Russia, and the USA in 2017–2022 (Table 1) and examined under a stereo microscope. The mites were collected using a minute pin, slide-mounted in a modified Berlese medium with Iodine [26], and cleared on a heating block at 90° C for 3–5 h. Some mites were stored in Eppendorf tubes filled with 96% ethanol and kept in a refrigerator (−25 °C) for DNA extraction. External morphology of the slide-mounted specimens was studied using conventional light microscopy (LM) using a Leica DM2500 and photographed with a ToupCam UCMOS09000KPB digital camera (Hangzhou ToupTek Photonics Co., Hangzhou, China). Morphological descriptions of all new species were based on phase contrast (PC) and differential interference contrast (DIC) LM observations. All measurements were obtained using ToupTek ToupView software x64 v. 4.11.19728.20211022 (http://www.touptek.com/download/showdownload.php?lang=en&id=33, accessed on 23 February 2023). They are given in the descriptions in micrometers (μm) in length except when stated otherwise. The measurements of females are based on the holotype, whereas the ranges (in brackets) are based on measurements of the paratypes and holotype. In the descriptions of all other instars, only ranges are given. Asterisks in descriptions mean "no variation". Terminology of eriophyoid morphology and classification of Eriophyoidea follow [23,27], respectively.

Statistical analyses. Measurements of 68 most used morphological traits in eriophyoid studies of 19 females from two populations of *Dicruvasates* mites, one from Egypt (n = 10) and the other from South Africa (n = 9), were analyzed using the Statistica 12.5 software package (TIBCO, Hamburg, Germany). A matrix of measurements was standardized, and a one-way multivariate analysis of variance (MANOVA) was used to examine the differences in morphological variation among the two groups of *Dicruvasates*. Multidimensional scaling (MDS) was performed to visualize differences among two mite groups, and general discriminant analysis (GDA, forward stepwise regression) was applied to determine the set of morphometrics that allows for the best discrimination between *Dicruvasates* spp.

Table 1. Collection data, DNA isolates, and GenBank accession numbers for sequences of three marker genes of *Dicruvasates*, *Echinacrus,* and *Phyllocoptes* mites obtained in this study.

Mite Species	Collecting Data	DNA Isolate	Cox1	28S D1D2	ITS1-5.8S-ITS2
Dicruvasates ngubani **n. sp.**	SOUTH AFRICA: Noorspoort farm, Steytlerville, Eastern Cape (33°17′44″ S, 24°21′34″ E), vagrant on bud scales and twigs of *Tamarix usneoides* E.Mey. ex Bunge (Tamaricaceae), 19 October 2022, coll. N.P. Ngubane-Ndhlovu and S. Situngu.	d371	PP190377	PP187378	PP187385
	SOUTH AFRICA: near Dwayka River, Western Cape (33°05′09″ S, 21°34′40″ E), vagrant on bud scales and twigs of *Tamarix usneoides* E.Mey. ex Bunge (Tamaricaceae), 19 October 2022, coll. N.P Ngubane-Ndhlovu and S. Situngu.	d9	–	–	PP187384
		c11	–	–	PP187382
		c12	–	–	PP187383
Dicruvasates tamaricis Abou-Awad and El-borolossy, 1995	EGYPT: Qalyubia governorate, 30°17′20.02″ N, 31°14′51.85″ E, on twigs of *Tamarix nilotica* (Ehrenb.) Bunge (Tamaricaceae), 9 March 2021, coll. A. Elhalawany.	d372	PP190378	PP187377	PP187381
Echinacrus septemcarinatus (Liro, 1941)	RUSSIA: Krasnodar Krai, Adler City District, near Krasnaya Polyana, right bank of river Mzymta, 43°39′21.9″ N, 40°09′23.0″ E, a vagrant on the lower leaf surface of *Frangula alnus* Mill. (Rhamnaceae), 10 July 2021, coll. P.E.Chetverikov.	d388	PP190375	PP187375	PP187388
	RUSSIA: Leningrad Oblast, Gatchina District, vil. Vyritza, 59°23′43.3″ N, 30°18′00.1″ E, a vagrant on the lower leaf surface of *Frangula alnus* Mill. (Rhamnaceae), 25 September 2017, coll. P.E.Chetverikov.	d383	PP190374	PP187376	PP187387
Phyllocoptes bilobospinosus Chetverikov et al., 2019	USA: Kansas, Garden City, right bank of Arkansas river, 37°57′24.9″ N 100°53′57.3″ W, on young shoots of *Tamarix ramosissima* Ledeb. (Tamaricaceae), 12 July 2018, coll. S. Zukoff.	d391	–	PP187380	PP187386
	RUSSIA: Crimea, Alushta Municipality, vil. Pryvetnoye, Kanaka Nature Reserve, roadside near the coast of Black Sea, 44°47′13′ N′, 34°39′01′ E′, on young shoots of *Tamarix tetrandra* Pall. ex Bieb. (Tamaricaceae), 3 June 2018, coll. P.E.Chetverikov.	d214	PP190376	PP187379	–

Confocal laser scanning microscopy. CLSM was used for detection of the anal secretory apparatus (ASA) of the mites. For this purpose, 37 ethanol-preserved adults and immatures of *Dicruvasates* spp. from South Africa and Egypt were mounted in Hoyer's medium [26] and investigated with the aid of a Spectral confocal and multiphoton system Leica TCS SP2 with objectives 40× N.A. 1.25–0.75 Oil CS HCX PL APO and 63^{x} N.A. 1.4–0.60 Oil lBL HCX PL APO using blue laser (405 nm). Image stacks were merged into maximum intensity projections (MIP) or volume rendering (VR) images with the aid of the reconstruction software ImageJ 1.54i [28] and Amira 5.3.2 [29].

Molecular phylogenetics. For molecular studies, we obtained partial sequences of three genes (*Cox1*, *28S*, and *ITS1-5.8S-ITS2*) of *Dicruvasates*, *Phyllocoptes*, and *Echinacrus* (Table 1) using our previously described methodology and protocols for DNA extraction, PCR, and sequencing [30] and combined them with selected sequences of Eriophyidae from GenBank. Sequences of the highly variable *ITS* region were used to validate the conspecificity of *Dicruvasates* from South Africa. All sequences were blasted against sequences of Eriophyidae from GenBank.

Two sequence datasets (*Cox1* and *28S*) were made for molecular phylogenetic analyses. For creating the *Cox1* dataset, we blasted sequence PP190377 of *Dicruvasates ngubani* **n. sp.** against Eriophyidae, filtered the resulting sequences of >50% coverage, added our new *Cox1* sequences (Table 1), and aligned the sequences in MAFFT [31,32] with defaults adjustments. Afterward, we removed (a) all duplicate sequences, (b) sequences containing indels and degenerate nucleotides (R, Y, N, etc.), (c) those which were notably shorter from

the 3′ and/or 5′ ends, and (d) all incorrectly identified sequences. The final *Cox1* alignment contained 355 sequences and 1158 nucleotide positions (386 amino acid positions). For creating the *28S* dataset, we blasted sequence PP187378 of *D. ngubani* **n. sp.** against Eriophyidae, filtered resulted sequences of >45% coverage, added our new *28S* sequences (Table 1), and aligned the sequences in MAFFT [31,32] using E-INS-i algorithm. Afterward, we removed the duplicate and incorrectly identified sequences and obtained the final *28S* alignment consisting of 207 sequences and 1152 nucleotide positions.

Two maximum likelihood (ML) analyses were performed in IQ-TREE 2.1.2 [33]. Models GTR+F+I+R7/GTR+F+I+I+R6 for *Cox1* (1st and 2nd positions)/28S gene evolution were selected using ModelFinder [34], as implemented in IQ-tree 2 based on the Bayesian information criterion. Branch support values were generated from the Ultrafast bootstrap approximation [35] with 10,000 bootstrap alignments, 10,000 maximum iterations, and a minimum correlation coefficient of 0.99. Values of a single branch test (SH-like approximate likelihood ratio test, SH-aLRT) with 10,000 replicates and Ultrafast bootstrap support (UFBS) were labelled on the ML trees. All trees were rooted with Nothopodinae (Eriophyidae).

3. Results

3.1. Systematics

Family Eriophyidae Nalepa, 1898

Subfamily Phyllocoptinae Nalepa, 1891

Tribe Phyllocoptini Nalepa, 1892

Genus *Dicruvasates* Abou-Awad and El-Borolossy, 1995

Abou-Awad and El-Borolossy 1995:145, Figure 1; Amrine et al. 2003: 107, 195, Figure 197.

Revised diagnosis. The body is somewhat fusiform; the gnathosoma is large, projecting obliquely downward. A prodorsal shield with a broad-based apically rounded frontal lobe superimposes over a subtriangular supracapitular plate (sensu [19], sometimes called "secondary lobe" of the prodorsal shield); scapular tubercles papilla-shape are situated notably ahead of the rear shield margin. Setae *sc* is directed upward. Dorsal opisthosoma have annuli bearing four longitudinal ridges (two subdorsal and two lateral) bearing spinules and/or small, subtriangular, or semilunar plates. The dorsal opisthosomal ridges start at the rear of the prodorsal shield margin and continue caudate, delimiting more or less distinct middorsal and lateral longitudinal furrows. The legs are with the usual series of setae, empodium simple, tarsal solenidion apically truncate, or a very small knob. All standard setae on the opisthosoma are present. A female genital coverflap is without longitudinal ribs. A short medial rod-like ridge is present on the ventral surface of telosome between the last ventral telosomal annulus and anal lobe.

Remarks. The original description of the type species and diagnosis of *Dicruvasates* report that "Anterior shield lobe . . . always ending in 2 spines, bifurcating over rostrum and subparallel to the chelicera". Our data suggest these "spines" are apodemes—the internal structures that are commonly seen in the basal part of palp in most slide-mounted eriophyoids as two dark lines, often forming a V-shape structure ([36], Figure 1, red arrow). We remove this artificial trait (two processes bifurcating over gnathosoma) from the diagnosis of *Dicruvasates*.

Type species: *Dicruvasates tamaricis* Abou-Awad and El-Borolossy, 1995

Host: Up to now, this genus has been recorded only from three species of the genus *Tamarix* (Tamaricaceae): *Tamarix nilotica* (Ehrenb.) Bunge, *T. usneoides* (new host, this paper), and *Tamarix* sp.

Number of species: Two.

Species included: *D. tamaricis* Abou-Awad and El-Borolossy, 1995 and *D. ngubani* **n. sp.** (this paper).

Distribution: Up to now, the genus *Dicruvasates* has been reported only from Egypt and South Africa; however, it may be distributed notably wider considering the natural distribution of the host plant genus *Tamarix* in Africa and Eurasia and numerous introductions of tamarisks worldwide.

***Dicruvasates tamaricis* AbouAwad and El-Borolossy, 1995 (Figures 2 and 3).**

Supplementary description. FEMALE (*n* = 10). The body is fusiform, 218–280, including gnathosoma, 60–87 wide, and 50–75 thick; yellow in life. **Gnathosoma** 35–45, projects obliquely downwards, *ep* 2–4, *d* 8–10, *v* 2–3, cheliceral stylets 25–32. **Prodorsal shield** is 35–39 long, including frontal lobe, 45–47 wide; sub-triangular with a broad-based frontal lobe 8–11, over gnathosomal base, apically rounded. The median and admedians lines are absent; submedian lines connect between scapular tubercles at the rear margin of the prodorsal shield, forming a U-shape. The scapular tubercles are 20 (18–23) apart, on the rear shield margin with diagonal axes, setae *sc* 15 (14–17), directed the setae diagonally inward to the rear.

Coxigenital area have faint granules, with 5 (4–5) semiannuli between coxae and genitalia, prosternal apodeme 4 (4–6); setae *1b* 11 (10–12), 12 (10–13) apart; *1a* 21 (20–23), 7 (6–8) apart; *2a* 29 (25–35), 26 (25–27) apart. **External genitalia.** Genital coverflap is subcircular, smooth, 15 (13–16) long, 19 (18–20) wide, *3a* 31 (28–35), 11 (11–12) apart. **Leg I** 30 (27–31), femur 10 (9–10), setae *bv* 14 (12–15); genu 5 (3–6), setae *l″* 22 (19–23); tibia 6 (5–6), setae *l′* 7 (6–8), located in the middle from the dorsal base; tarsus 6 (5–6); empodium simple 8 (7–8), 7/8-rayed, tarsal solenidion *ω* tapered, 9 (9–10), setae *ft′* 19 (17–21), *ft″* 22 (21–25), *u′* 7 (7–8). **Leg II** 26 (25–27), femur 9 (9–10), *bv* 14 (12–18); genu 4 (4–5), *l″* 10 (9–10); tibia 4 (4–5); tarsus 4 (4–5) empodium simple 10 (9–10), 7/8-rayed, *ω* tapered, 9 (8–9), *ft′* 15 (13–17), *ft″* 22 (20–23), *u′* 6 (5–7).

Opisthosoma has 41 (40–42) dorsal semiannuli and 54 (53–56) ventral semiannuli. Dorsal annuli have spines bearing ridges with a broad base; with four longitudinal ridges, two sub-dorsal, and two lateral ridges started from the rear of shield margin and continuing caudate; and middorsal furrow scarcely apparent, narrow, the last third dorsal annuli with pointed microtubercles. Ventral annuli has round microtubercles placed near the rear margin of the annuli; and the last 12–14th ventral annuli bearing linear elongate microtubercles. Setae *c2* 45 (30–48), 45 (44–52) apart, on annulus 10 (9–10) from coxae II; *d* 40 (36–43), 37 (33–40) apart, on annulus 19 (17–19); *e* 27 (22–30), 15 (14–15) apart, on annulus 38 (36–38); *f* 30 (28–32), 21 (20–23) apart, on the sixth annulus from the rear, *h1* 3 (2–4), *h2* 53 (49–63).

MALE (*n* = 4). Similar to the female, the body is fusiform, 187–190, including gnathosoma, 49–54 wide and 46–62 thick, yellow in life. **Gnathosoma** 30–35, cheliceral stylets 25–32, *ep* 2–3, *d* 7–9, *v* 2–3. **Prodorsal shield** pattern similar to that of female, 35–40 long including frontal lobe, 38–44 wide, frontal lobe 8–9, rounded; scapular tubercles 15–16 apart, on rear shield margin with diagonal axes, setae *sc* 11–15, directed the setae diagonally inward to the rear. **Coxigenital area** coxae I with granules, prosternal apodeme 4–5; *1b* 9–10, 10–12 apart; *1a* 17–20, 6–7 apart; *2a* 26–30, 22–24 apart. **Genital area** 10–13 long, 15–17 wide, *3a* 27–34, 12–13 apart. **Leg I** 25–28, femur 9–10, *bv* 13–15; genu 3–5, *l″* 17–21; tibia 5–7, *l′* 6–8; tarsus 5–6; empodium simple 7–8, 7/8-rayed, *ω* 8–9 tapered, *ft′* 19–20, *ft″* 21–23, *u′* 7–8. **Leg II** 23–26, femur 8–9, *bv* 12–13; genu 3–5, *l″* 8–10; tibia 3–4; tarsus 4–5; empodium 7–8, 7/8-rayed, *ω* 8–10 tapered, *ft′* 13–15, *ft″* 21–22, *u′* 6–7.

Opisthosoma with 38–42 dorsal semiannuli and 49–53 ventral semiannuli, microtubercles on dorsal and ventral annuli shape similar to that of female. Setae *c2* 29–35, 43–48 apart, on annulus 8–9 from coxae II; *d* 30–44, 31–36 apart, on annulus 15–17; *e* 26–35, 13–15 apart, on annulus 30–35; *f* 27–30, 15–19 apart, on the sixth annulus from the rear, *h1* 2–3, *h2* 45–60.

NYMPH (*n* = 3). Body fusiform, 162–178 including gnathosoma, 45–50 wide and 50–60 thick, yellow in life. **Gnathosoma** 27–31, cheliceral stylets 20–22, *ep* 1–2, *d* 5–6, *v* 1–2. **Prodorsal shield** smooth, 35–42 long including frontal lobe, 35–40 wide, frontal lobe 5–6, rounded; scapular tubercles ahead of rear shield margin, 10–11 apart, *sc* 8–10, directed upward. **Coxigenital area** is smooth, prosternal apodeme 3–4; *1b* 6–7, 34–5 apart; *1a* 12–13, 5–6 apart; *2a* 18–20, 12–13 apart; *3a* 15–17, 4–5 apart. **Leg I** 21–23, femur 7–8, *bv* 9–10; genu 3*, *l″* 14–16; tibia 4*, *l′* 3–4; tarsus 4–5; empodium simple 5–6, 6-rayed, *ω* 6–7 tapered, *ft′* 14–15, *ft″* 17–19, *u′* 3–4. **Leg II** 17–18, femur 6–7, *bv* 9–10; genu 3*, *l″* 5–7; tibia 3*; tarsus

4–5; empodium 5–6, 6-rayed, *ω* 6–7 tapered, *ft′* 10–11, *ft″* 13–14, *u′* 3–4. **Opisthosoma** with 45–47 dorsal semiannuli and 49–53 ventral semiannuli, dorsal microtubercles smooth, and ventral annuli with pointed microtubercles on rear annuli. Setae *c2* 25–31, 39–41 apart, on annulus 8–9 from coxae II; *d* 27–31, 30–31 apart, on annulus 19–20; *e* 17–21, 10–11 apart, on annulus 40–41; *f* 17–20, 11–12 apart, on the sixth annulus from the rear, *h1* 1–2, *h*2 23–25.

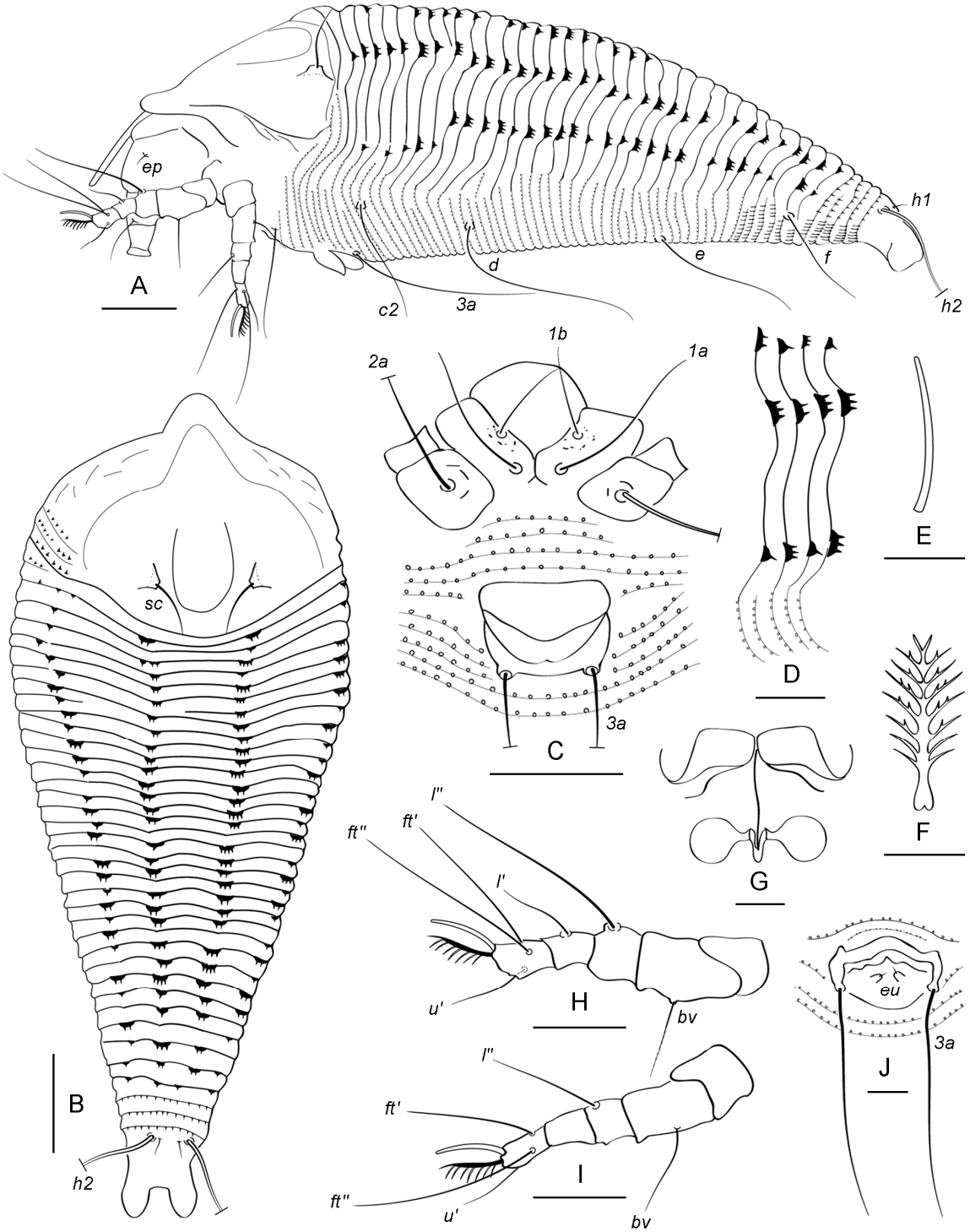


Figure 2. Drawings of *Dicruvasates tamaricis* AbouAwad and El-Borolossy, 1995. (**A**,**B**) Lateral (**A**) and dorsal (**B**) view of female; (**C**) Female coxigenital area; (**D**) Lateral opisthosomal annuli; (**E**) Tarsal solenidion I; (**F**) Empodium I; (**G**) Female internal genitalia; (**H**) Leg I; (**I**) Leg II, (**J**) Male genital area. Scale bar: (**A**–**C**) 20 μm; (**D**) 15 μm; (**E**–**G**,**J**) 5 μm; (**H**,**I**) 10 μm. Drawings—A. Elhalawany.

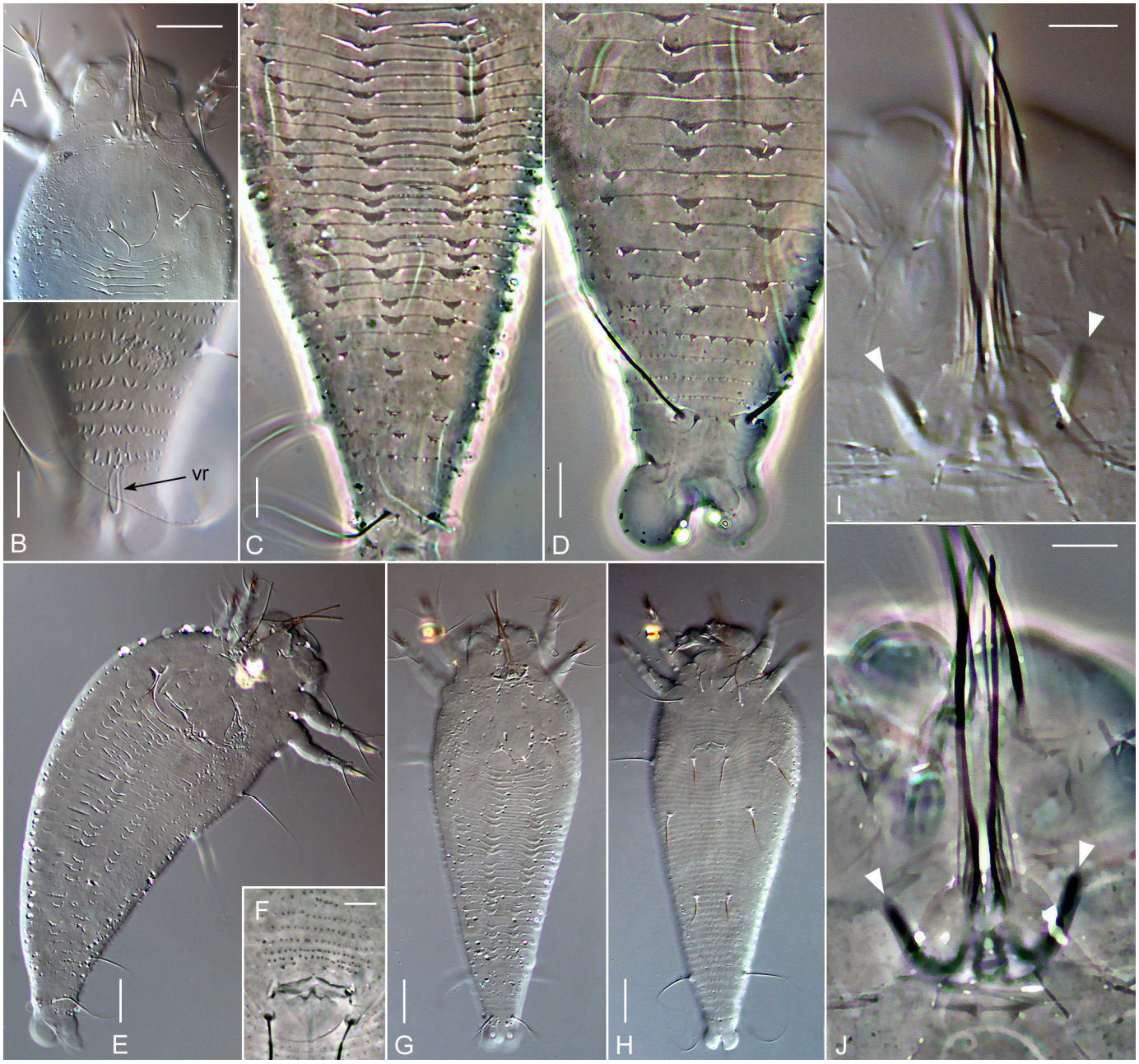


Figure 3. DIC LM (**A**,**B**,**E**,**G**,**H**,**I**) and PC LM (**C**,**D**,**F**,**J**) microphotographs of *Dicruvasates tamaricis* AbouAwad and El-Borolossy, 1995. (**A**) Prodorsal shield; (**B**) Ventral view of telosoma and ventral ridge (vr); (**C**,**D**) Dorsal view of rare part of opisthosoma in female (**C**) and male (**D**); (**E**) Semilateral view of female; (**F**) Male genital area; (**G**,**H**) Dorsal (**G**) and ventral (**H**) view of male; (**I**,**J**) Two microphotographs showing palpal apodemes (white arrowheads). Scale bars: (**A**). 20 µm; (**B**–**D**,**F**,**I**,**J**). 5 µm; (**E**,**G**,**H**). 15 µm. Photos—P.E.Chetverikov.

Host plant in Egypt. *Tamarix nilotica* (Ehrenb.) Bunge (Tamaricaceae).

Relation to the host plant. Vagrant on leaves and twigs.

Distribution. Up to now, this species has been found only in Egypt.

Materials examined. Fifteen females, six males, three nymphs, and four larvae on five slides (slide no. EGYErio58.1–58.5), from *Tamarix nilotica*, were deposited in the Plant Protection Research Institute collection, Giza, Egypt, 25 May 2022, Qalyubia governorate (30°17′20.02″ N, 31°14′51.85″ E). Ten females and seven males on ten slides (slide no. EGYErio58.8–58.17), Mariotya city (30°01′25.51″ N, 31°81′22.94″ E), Giza gover-

norate, Egypt, 15 January 2024, deposited at Plant Protection Research Institute collection, Giza, Egypt. Two slides (slides no. EgTT17-18), 9 March 2021, Qalyubia governorate (30°17′20.02″ N, 31°14′51.85″ E), deposited at UNIBA, Italy. Four slides with the same data were deposited at the Zoological Institute of the Russian Academy of Sciences (ZIN RAS), Russia. Two slides, Benha city (30°26′4.62″ N, 31°11′24.22″ E), Qalyubia governorate, Egypt, 15 March 2021, deposited at the College of Agriculture and Forestry, West Virginia University (WVU), USA. Four females on two slides (slide no. EGYErio 58.6–58.7), Mariotya city (30°01′25.51″ N, 31°81′22.94″ E), Giza governorate, Egypt, 20 April 2022, deposited at ESAM, Egypt. All materials were collected by A. Elhalawany.

Remarks. In comparison to specimens of *D. tamaricis* described by AbouAwad and El-Borolossy [21] from the El-Fayum governorate in Egypt, the specimens of the current study were collected from Giza and Qalyubia governorates in Egypt, the distance between the two localities is approximately 100 km. The specimens of the current study have longer (218–280 vs. 157 long) and wider (60–87 vs. 53 wide) bodies.

***Dicruvasates ngubani* n. sp.—**Figures 4–6**.**

FEMALE (*n* = 9). The body is fusiform, 194 (146–194) long, including the pedipalp, 58 (50–68) wide, of white to yellowish in life. **Gnathosoma** is 22 (8–23) long, projecting downwards antapical seta *d* 7 (6–8), basal setae *ep* 5 (4–6), apicoventral setae *v* 2 (2–3), cheliceral stylets 19 (16–26) long. **Prodorsal shield** is 42 (36–45) long with blunt subtriangular frontal lobe 6 (5–8) lying above a rounded subtriangular supracapitular plate (sensu [19]), 50 (45–52) wide; prodorsal shield ornamentation weak, short indistinct median line or cuticle fold was observed in several specimens (*n* = 3), usually a pair of obscure incomplete admedian lines present between tubercles of scapular *sc* setae, they curve outwards from rear, then inwards; epicoxal area with 2–3 irregular broken lines (may be absent) and sparse microtubercles. Scapular tubercles papilla–shaped, setae *sc* 12 (10–13), 20 (17–20) apart, directed inwards (in dorsoventrally oriented mites), upward (in lateral mites) or backward (in distorted or over–flattened mites).

Coxisternae I and II have small microtubercles and faint ridges; anterolateral setae on coxisternum I *1b* 12 (7–12) long and 12 (10–13) apart; proximal setae on coxisternum I *1a* 18 (17–23) long and 7 (7–9) apart; coxisternum II smooth proximal setae *2a* 30 (35–47) long and 25 (23–27) apart; prosternal apodeme weak, 4 (3–4); 2 (2–3) incomplete and 1 (1–2) complete coxigenital annuli between coxisternae II and epigynium. **Genital coverflap** is 13 (11–18) long, 18 (15–21) wide, with two short transverse arc-shaped ridges in middle part; proximal setae on coxisternum III *3a*, 56 (39–56), 12 (11–14) apart. **Internal genitalia.** Anterior genital apodeme trapezoidal, oblique apodeme distinct, short spermathecal tubes short, about 2, directed laterad; spermathecae subspherical, about 5 in diameter, with tiny spermathecal processus.

Leg I 26 (24–26), femur 9 (7–10), basiventral femoral seta *bv* 13 (11–15); genu 4 (3–4), antaxial genual seta *l″* 17 (17–20); tibia 5 (5–6), paraxial tibial seta *l′* 8 (6–9) tarsus 5 (5–6); tarsal empodium simple 7 (7–8), 7–rayed, tarsal solenidion *ω* slightly curved, with very small subspherical knob, 7 (7–8), paraxial fastigial tarsal seta *ft′* 23 (18–24), antaxial fastigial tarsal seta *ft″* 17 (17–21), paraxial unguinal tarsal seta *u′* 8 (6–10). **Leg II** 26 (23–26), femur 8 (8–10), seta *bv* 15 (11–15); genu 4 (3–4), seta *l″* 11 (9–11); tibia 4 (4–5); tarsus 5–6; tarsal *em* simple 7 (7–8), 7–rayed, *ω* slightly curved 7 (7–8), seta *ft′* 22 (20–24), seta *ft″* 10 (7–10), seta *u′* 4 (4–7).

Opisthosoma with 39 (36–39) broad dorsal annuli and 51 (46–53) ventral annuli. Dorsal annuli form four longitudinal ridges bearing acuminate, subtriangular spiculate plates, and flanking medial and lateral furrows. First 7 (7–8) dorsal annuli adjacent to rear margin of prodorsal shield medially with small rounded microtubercles. Ventral annuli with small rounded microtubercles anterior to tubercles of setae *e* and with more elongated and pointed microtubercles beyond setae *e*. Lateral setae *c2* 36 (30–40), 51 (45–53) apart, on annulus 7 (5–9); ventral setae *d* 52 (45–68), 36 (34–39) apart, on annulus 17 (17–18); ventral

setae *e* 36 (28–36), 17 (16–19) apart, on annulus 30 (29–31); ventral setae *f* 33 (32–38), 23 (16–24) apart, on 6 (5–6) annulus from rear. Setae *h1* 4 (3–5); setae *h2* 70 (60–70).

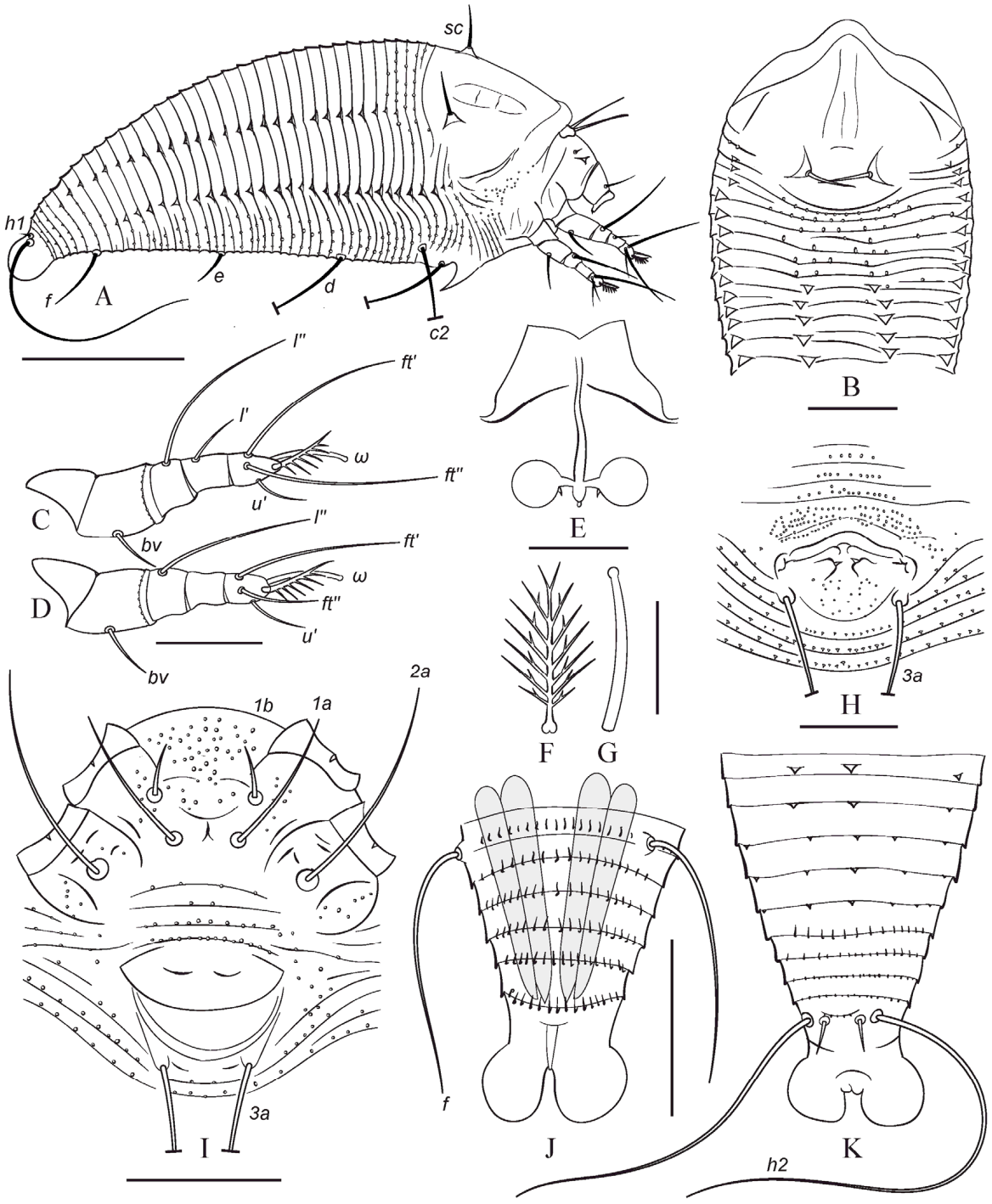


Figure 4. Drawings of *Dicruvasates ngubani* **n. sp.** (**A**) Lateral view of entire mite; (**B**) Prodorsal shield and anterior part of dorsal opisthosoma; (**C**,**D**) Legs I and II; (**E**) Female internal genitalia; (**F**,**G**) Tarsal empodium I and solenidion I; (**H**) Male external genitalia; (**I**) Female coxigenital area; (**J**,**K**) Ventral and dorsal view of telosome. Note: traces of two paired anal glands under ventral telosomal cuticle are shown in (**J**). Scale bar: (**A**). 50 μm; (**B**,**I**–**K**). 20 μm; (**C**–**E**). 10 μm; (**F**,**G**). 5 μm; (**H**). 15 μm. Drawings—P.E.Chetverikov.

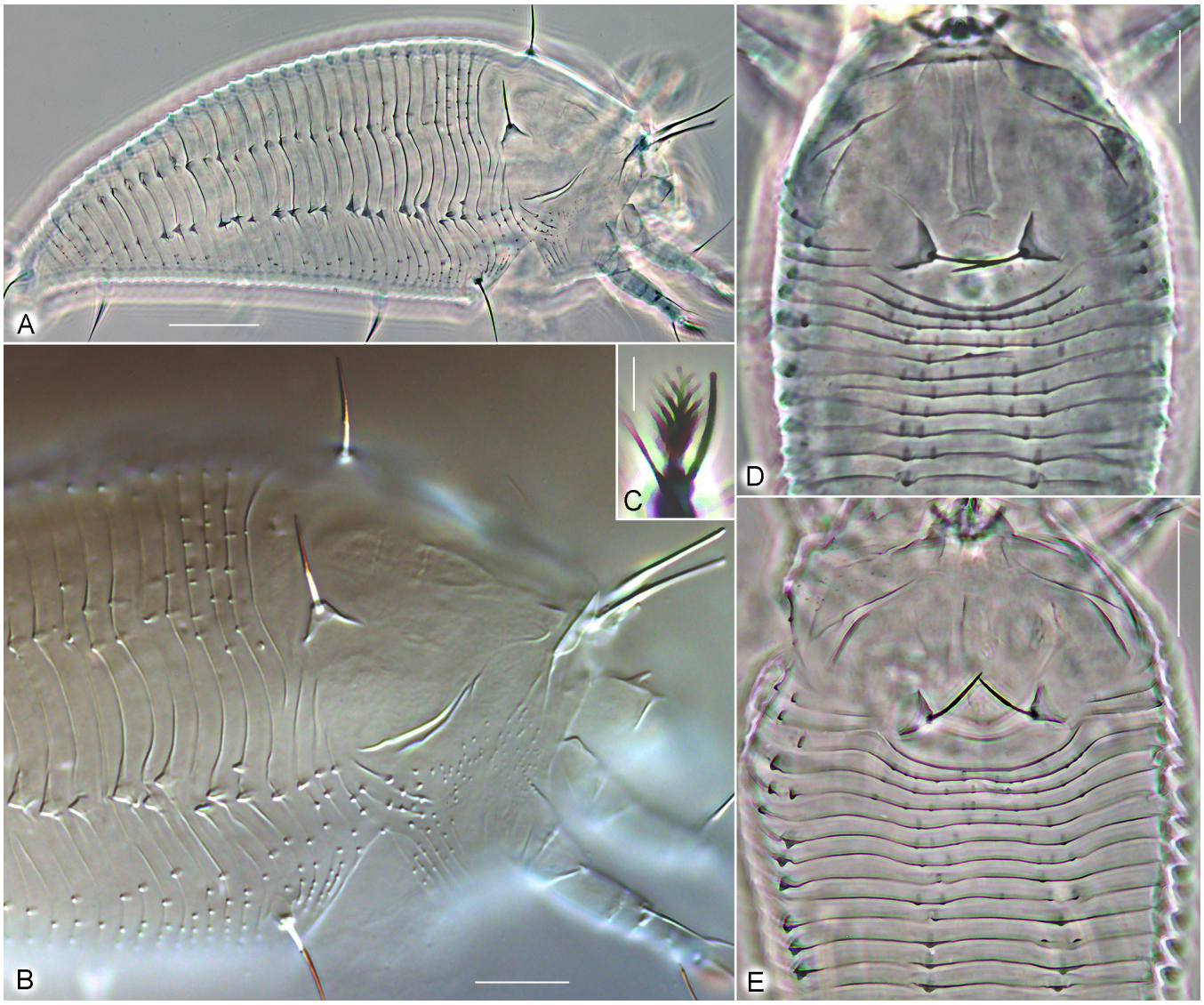


Figure 5. PC LM (**A**,**C**–**E**) and DIC LM (**B**) microphotographs of *Dicruvasates ngubani* **n. sp.** (**A**) Lateral view of entire female; (**B**) Lateral view of anterior part of body of a female; (**C**) Empodium I and tarsal solenidion I; (**D**,**E**) Male (**D**) and female (**E**) prodorsal shield and anterior part of dorsal opisthosoma. Scale bar: (**A**,**D**,**E**) 20 μm; (**B**) 10 μm; (**C**) 3 μm. Photos—P.E.Chetverikov.

MALE (*n* = 2). Similar to the female, the body is fusiform, white or yellowish, 149–167 long. **Gnathosoma** 24, cheliceral stylets 18–27 long, setae *d* 6–7. The **prodorsal shield** pattern is similar to that of the female, 33–40 long, 43–47 wide, frontal lobe 6 long. Scapular tubercles 14–19 apart, setae *sc* 11–20. **Coxigenital area.** Prosternal apodeme 4–6 long; setae *1b* 6–9, 9–10 apart; setae *1a* 17–19, 6–7 apart; setae *2a* 19–23, 18–19 apart. **Leg I** 24, femur 8–9, seta *bv* 13–14; genu 4–4 seta *l″* 12–16; tibia 5–6, tibial setae 7–9; tarsus 5–5; empodium simple *em* 6–7, 7-rayed, tarsal solenidion *ω* 7–8 slightly curved, seta *ft′* 20–21, seta *ft″* 21–22, setae *u′* 6–7. **Leg II** 24–26, femur 7–8, seta *bv* 14–13; genu 4*, seta *l″* 10*; tibia 4*; tarsus 5*; empodium 7*, 7-rayed, solenidion *ω* 8* slightly curved, seta *ft′* 7*, seta *ft″* 21–22, seta *u′* 6–7. **Opisthosoma** with 37–38 dorsal and 45–46 ventral annuli microtuberculate, similar to females. Lateral setae *c2* 28–32, 49–51 apart, on annulus 4–8; ventral setae *d* 53–55, 33–36 apart, on annulus 13–17; setae *e* 25–29, 10–11 apart, on annulus 13–17; setae *f* 23–27, 23 apart, on 5–6 annulus from rear. Setae *h1* 2–3; setae *h*2 broken. **External genitalia** 13–14 long, 15 wide, surface between eugenital setae with small rounded microtubercles, setae *3a* 39–50, 15 apart.

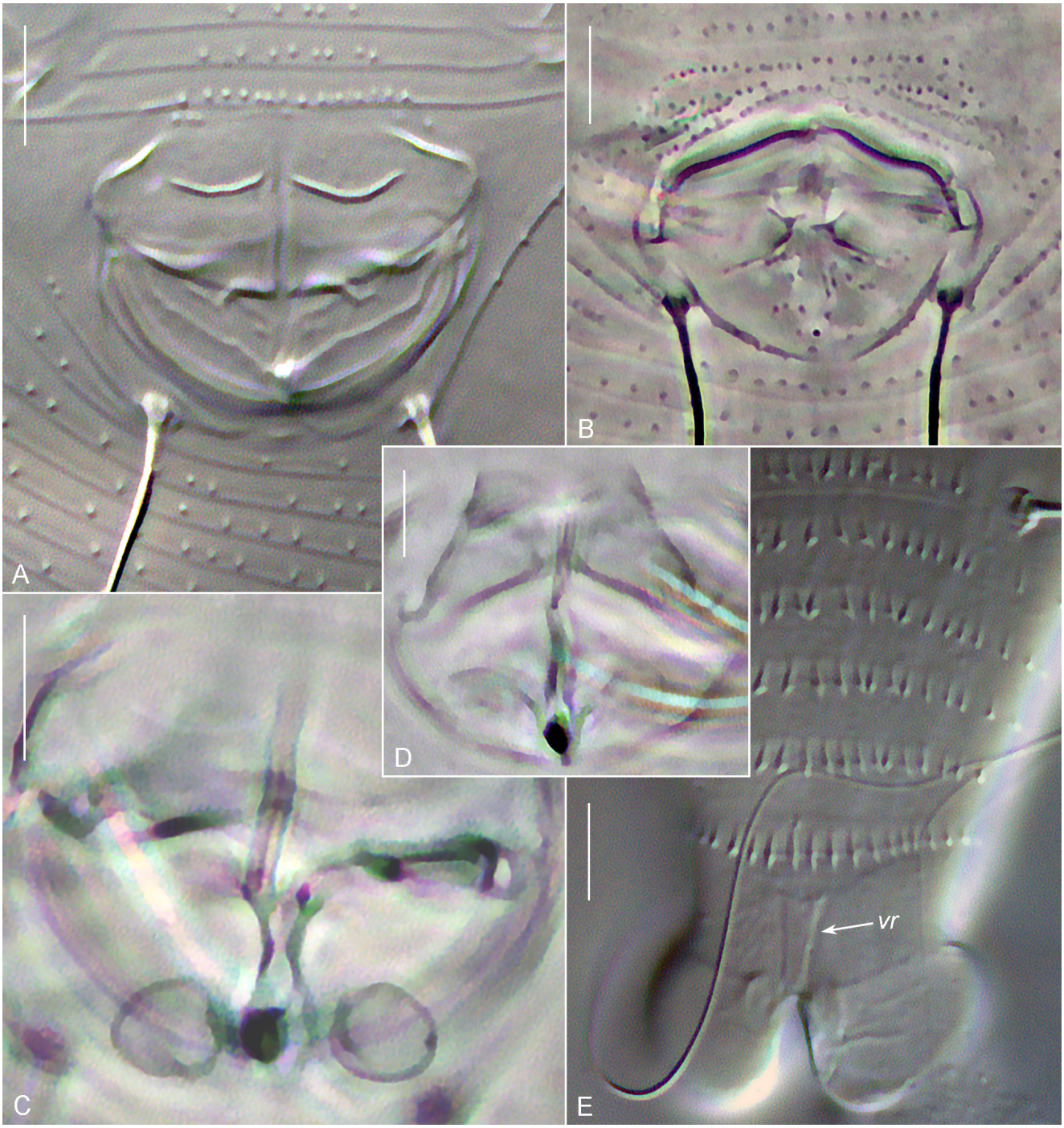


Figure 6. DIC LM (**A**) and PC LM (**B**–**E**) microphotographs of *Dicruvasates ngubani* **n. sp.** (**A**,**B**) Female (**A**) and male (**B**) external genitalia; (**C**,**D**) Two images of female internal genitalia showing spermathecae (**C**) and anterior genital apodeme (**D**); (**E**) Dorsal view of telosome and caudal lobes. Note: arrow in (**E**) indicates ventral ridge (*vr*). Scale bar—5 µm. Photos—P.E.Chetverikov.

Host plant. *Tamarix usneoides* E.Mey. ex Bunge (Tamaricaceae).

Relation to the host plant. Vagrant, probably causes rusting of twigs.

Etymology. The name of the new species, *ngubani*, is derived from the surname of the second author of this paper.

Type locality. The mites were found near Noorspoort farm, Steytlerville, Eastern Cape (33°17′44″ S, 24°21′34″ E).

Type material. It comprises the holotype female (slide X2023-01), paratype females (slides X2022-03 and X2023-01), and males (slides X2023-01) from twigs of *Tamarix usneoides* near Noorspoort on 17 February 2023 coll. N. Ngubane-Ndhlovu and S. Situngu. Type material has been deposited at the Department of Agriculture, Land Reform and Rural Development at Plant Quarantine Station in Stellenbosch, South Africa. Additionally, some paratype material has been deposited at the Zoological Institute of the Russian Academy of Sciences (ZIN RAS), Russia.

Additional material. The same host and collectors, slides X2021-01, X2021-02, and X2021-04 were collected on 26 February 2021 near Dwayka River, Western Cape, South Africa (33°05′09″ S, 21°34′40″ E).

Statistical analysis. The one-way MANOVA of the two populations of *Dicruvasates* (*D. tamaricis* from Egypt and *D. ngubani* **n. sp.** from South Africa) revealed significant differences in the morphological variation of 68 morphological traits (Wilks' Lambda = 0.000202, $p < 0.05$). MDS showed two well-separated clouds of dots corresponding to different species of *Dicruvasates* (Figure 7). GDA forward stepwise regression determined a set of 14 discriminating variables; among them, two variables (length of *ft*″ II and length of *ft*′ II) have the largest standardized regression coefficients and contribute most to the prediction of group membership [37].

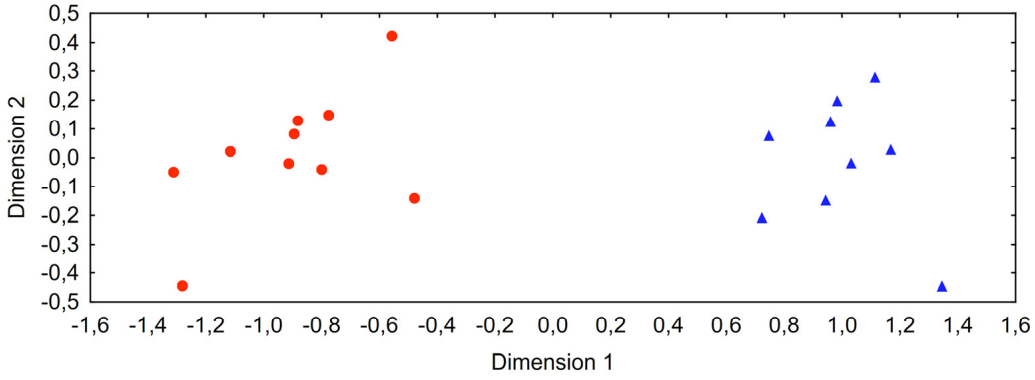


Figure 7. Graphical representation of the results of MDS analysis showing the distribution of *Dicruvasates tamaricis* (red circles) and *D. ngubani* n. sp. (blue triangles) specimens in the spaces of two dimensions (Stress = 0.0345297).

Differential diagnosis. The new species is very close to *Dicruvasates tamaricis* Abou-Awad and El-Borolossy, 1995. Contrary to the mites from the studied Egyptian population of *D. tamaricis*, in the new species, the small plates on the opisthosomal ridges are triangular and devoid of tiny marginal denticles (semilunar and denticulate in *T. tamaricis*), first 7–8 dorsal annuli adjacent to the rear margin of prodorsal shield medially with small rounded microtubercles (no microtubercles in *T. tamaricis*). The two species also notably differ in the lengths of leg setae *ft*″ II and *ft*′ II: in the new species, *ft*″ II is 7–10 (20–23 in *D. tamaricis*), and *ft*′ II is 20–24 (13–17 in *D. tamaricis*). Finally, these two *Dicruvasates* species have been collected in regions of Africa (Egypt and South Africa) remote in location and from the hosts that have non-overlapping distribution areas [21]: *Tamarix nilotica* (Ehrenb.), the host of *D. tamaricis*, is common in arid parts of North Africa and the Middle East, whereas *Tamarix usneoides*, the host of *D. ngubani* **n. sp.** grows in semi-deserts and karroid areas in southern Africa, ranging from Angola through Namibia to the Cape Provinces of South Africa (Figure 1).

Remarks. According to our observations in South Africa, *Dicruvasates ngubani* **n. sp.** coexists with *Aceria benhaiensis* Situngu et al., 2023 and *A. noorspoortiensis* Situngu et al., 2023 and is a common inquiline in the galls caused by *A. noorspoortiensis* on *Tamarix usneoides*.

Confocal microscopy. Most slides that were made for the CLSM study contained partially cleared specimens of *Dicruvasates*, which is typical when slide-mounting eriophyoids were kept in ethanol [38]. Under CLSM in the adults and immatures with incompletely

dissolved internal tissues, we observed outlines of anal glands. They were seen as dark spots surrounded by autofluorescing material, presumably consisting of remnants of the parenchymatous mite fat body, ovarium, and midgut (Figure 8G). In addition to the anal glands, which are soft tissue, we also observed the typical cuticle-lined elements of the anal secretory apparatus (ASA, sensu [20]): the paired gland ducts, dorsal and ventral sacs, and rectal tube. All these structures were observed in females, males, and, for the first time, in immatures. In larvae, the ASA is less developed, but in nymphs, it is almost the same size as in adults (Figure 8A vs. Figure 8B–F).

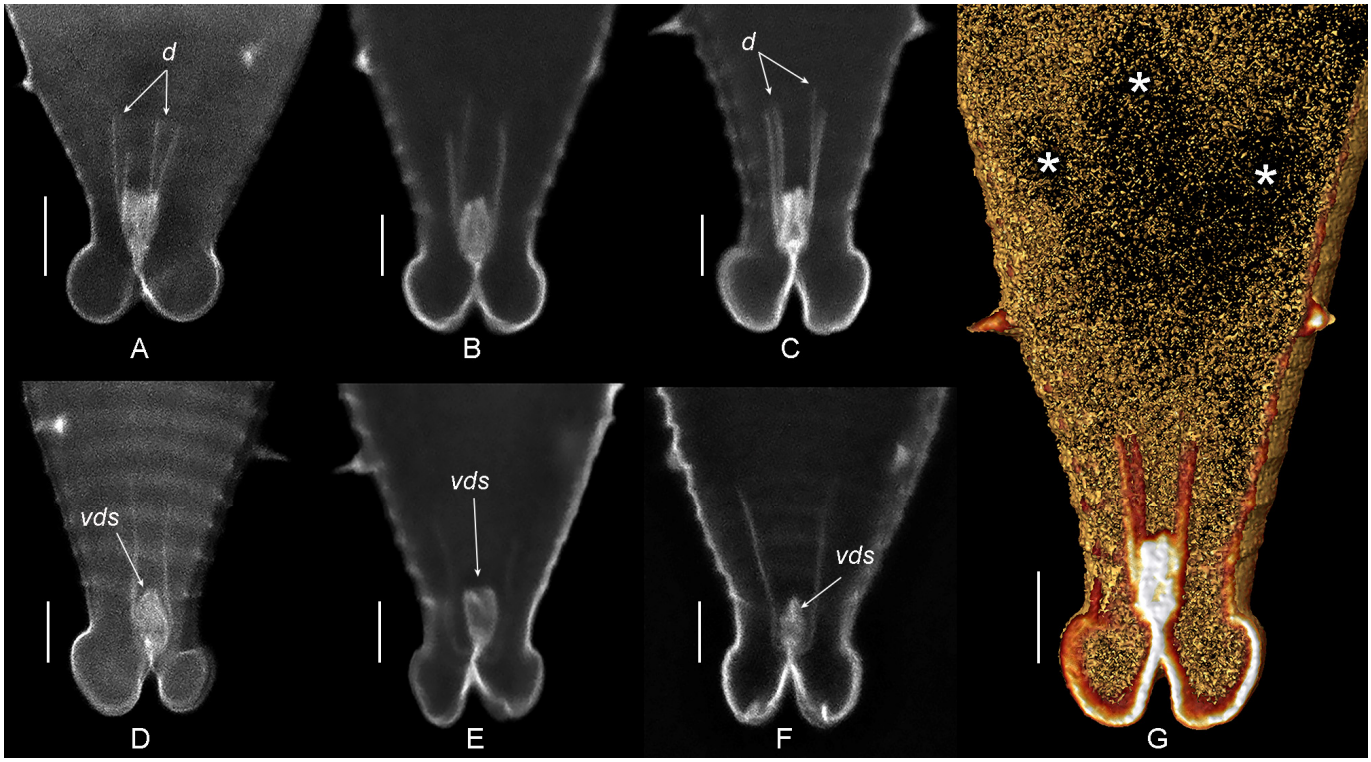


Figure 8. MIP (**A**–**F**) and VR (**G**) CLSM images of anal secretory apparatus in nymph (**A**), males (**B**,**C**), and females (**D**–**G**) of *Dicruvasates ngubani* **n. sp.** Notations: *d*—paired gland ducts, *vds*—ventral and dorsal sacs. Asterisks in (**G**) indicate anal glands. Scale bar = 10 μm. CLSM images—P.E.Chetverikov.

***Genus Echinacrus* Keifer, 1966**

=*Pentamerus* Roivainen, 1951, preoccupied by *Pentamerus* Sowerby, 1813

Remarks. Özdikmen [39] synonymized eriophyid genera *Pentamerus* Roivainen, 1951 and *Echinacrus* Keifer, 1966, showed that *Pentamerus* Roivainen, 1951 was preoccupied by *Pentamerus* Sowerby, 1813 and transferred all eriophyid species from *Pentamerus* Roivainen, 1951 to *Echinacrus* Keifer, 1966.

Diagnosis. Body fusiform, prodorsal shield with broad-based apically rounded frontal lobe, dorsal tubercles situated on rear shield margin directing setae *sc* divergently backward, ventral opisthosomal annuli distinctly more numerous than dorsal annuli, all typical leg and opisthosomal setae present, empodium simple, gnathosomal seta *d* non-bifurcated. Opisthosoma has five longitudinal ridges: one mid-dorsal ridge, two lateral ridges, and two sub-lateral ridges. The ridges bear spines, subtriangular plates, or narrow denticulate plates. Live mite with wax bands along the opisthosomal ridges.

Type of species: *E. septemcarinatus* (Liro, 1941).

Species included (*n* = 5): *E. acaciae* (Smith-Meyer, 1989), *E. halawanii* Elhalawany 2013, *E. meliae* (Kuang-Haiyuan, 1998), *E. psophocarpi* (Chandrapatya, 1992), *E. septemcarinatus* (Liro, 1941).

Hosts: Rhamnaceae (g. *Frangula*), Fabaceae (g. *Acacia*), and Meliaceae (g. *Melia*).

Relation to hosts: Vagrants cause no visible damage.
Distribution: North America, Europe, Asia, Africa [24,25].

***Echinacrus septemcarinatus* (Liro, 1941)—Figure 9.**
Epitrimerus septemcarinatus Liro, 1941:22, Figure 15
Pentamerus septemcarinatus (Liro, 1941) Roivainen 1951:52, Figure 23; Ripka 2007:103, Ripka 2008:158, Roivainen (1947):35, 1951:53, Figure 23, Farkas 1965:121, Figure 90, Skoracka, Lewandowski and Boczek 2005:118.

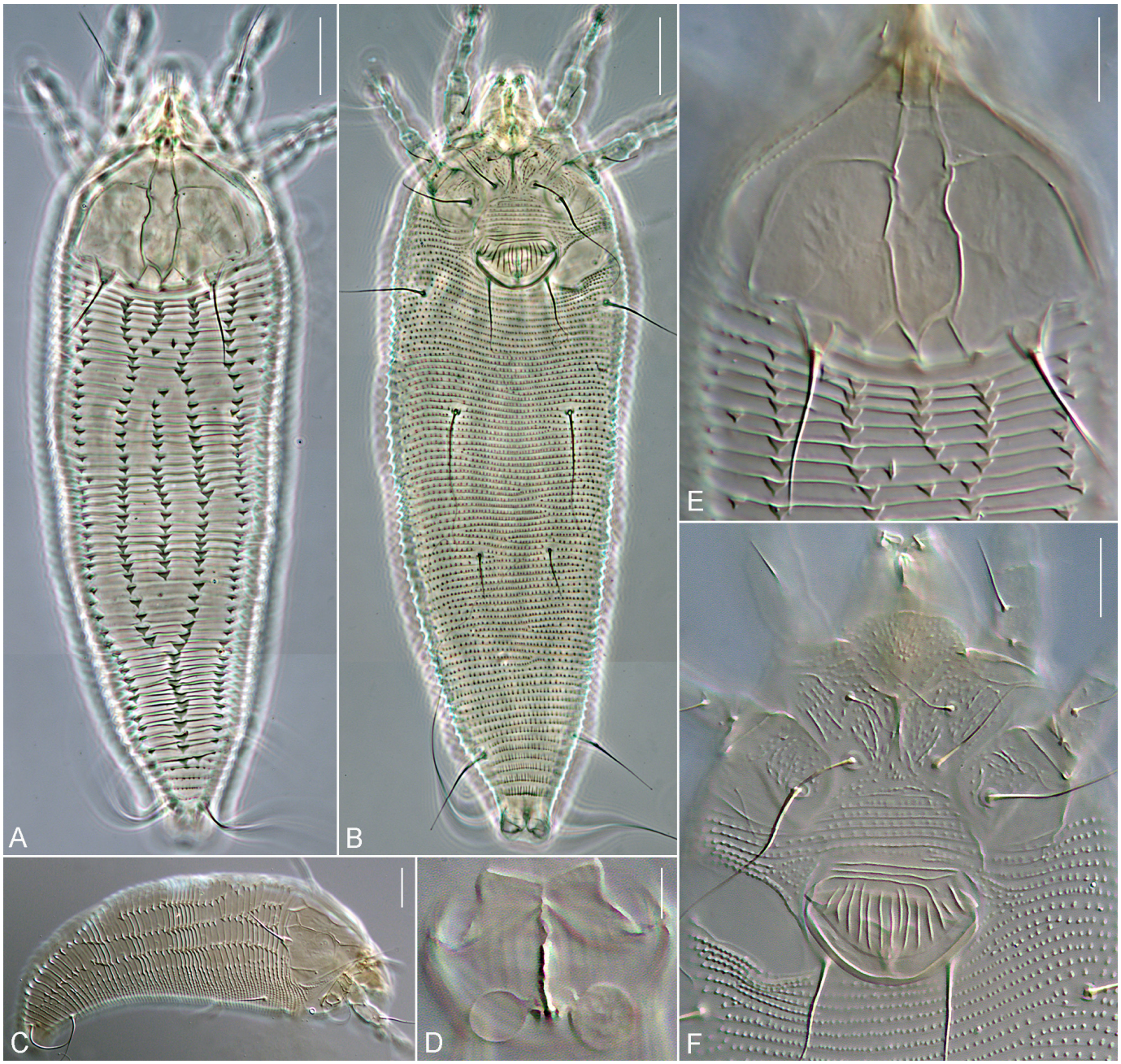


Figure 9. PC LM (**A**,**B**) and DIC LM (**C**–**F**) microphotographs of *Echinacrus septemcarinatus* (Liro 1941) from Vyritza (North-West Russia). (**A**) Dorsal view of female; (**B**) Ventral view of female; (**C**) lateral view of female; (**D**) Female internal genitalia; (**E**) Prodorsal shield; (**F**) Coxigenital area. Scale bar: (**A**,**B**) = 20 µm; (**C**–**F**) = 10 µm; (**D**) = 5 µm. Photos—P.E.Chetverikov.

Echinacrus septemcarinatus (Liro, 1941) Özdikmen (2008):217, 222; Elhalawany 2013:8.

Numerous females, males, and immatures were collected in Russia: Leningrad Oblast, Gatchina District, vil. Vyritza, 59°23′43.3″ N, 30°18′00.1″ E, a vagrant on the lower leaf surface of *Frangula alnus* Mill. (Rhamnaceae), on 22 July 2016, 25 September 2017, 19 and 26 May 2018, coll. P.E.Chetverikov; in Krasnodar Krai, Adler City District, near Krasnaya Polyana, right bank of river Mzymta, 43°39′21.9″ N, 40°09′23.0″ E, a vagrant on the lower leaf surface of *Frangula alnus* Mill. (Rhamnaceae), alder buckthorn, 10 July 2021, coll. P.E.Chetverikov.

3.2. Blast Search Results

No *Cox1* sequences of *Dicruvasates* are present in Genbank. Blastn/Blastx searches for our *Cox1* sequences of *Dicruvasates* (PP190377, PP190378) returned various sequences of Anthocoptinae (e.g., *Aculus amygdali*, *Aculops* sp., *Anthocoptes thymi*) with 74%–80%/90%–95% identity. Blastn/Blastx searches for both our *Cox1* sequences of *Echinacrus septemcarinatus* (PP190374 and PP190375) returned sequences MZ483149/UOK11147 of *E. rhamnicrocerae* from China as the best hits with 90.43%–97.30%/99.52% identity. Blastn/Blastx searches for the new *Cox1* sequence PP1900376 of *Tamarix*-associated mite species *Phyllocoptes bilobospinosus* from Crimea returned sequences OR133633/WIW35252 of *Aceria benhaiensis* from *Tamarix usneoides* from South Africa with 76.86%/89.1% identity and 99% coverage.

No *28S* sequences of *Dicruvasates* and *Echinacrus* are present in GenBank. Blast searches for two of our *28S* sequences (PP187375 and PP187376) of *E. septemcarinatus* returned sequence KT779210 (*Aceria artemisiifoliae*) when sorted by identity and MN594271 (*Aceria litchii*) when sorted by E-value. Blast searches for our *28S* sequences of *Dicruvasates* from African tamarisks (PP187377, PP187378) returned as best hits the sequences of eriophyids also associated with *Tamarix*: MK408624 (*Phyllocoptes bilobospinosus*) when sorted by identity and OR139570 (*Aceria noorspoortiensis*) when sorted by E-value. Blast searches for our *28S* sequences of *Phyllocoptes bilobospinosus* from Crimea (PP187379) and the USA (PP187380) identified conspecific sequence MK408624 as the closest one with 100% and 94.77% identity (correspondingly) and 56% coverage.

ITS sequences of *Dicruvasates ngubani* **n. sp.** from different populations from South Africa were identical, confirming their conspecificity. Interspecific and intergeneric variability of ITS sequences was very high, so it was not possible to align them unambiguously, which was expected considering the high variability of ITS1,2 in eriophyoids.

3.3. Molecular Phylogenetic Analyses

Both *Cox1* and *28S* single gene analyses produced incompletely resolved trees of Eriophyidae. In all analyses, a highly supported clade (Tam-clade, Figure 10) comprising sequences of *Dicruvasates*, *Phyllocoptes*, and *Aceria* associated with the plant genus *Tamarix* was recovered. Phylogenetic relations within the Tam-clade were poorly resolved. All sequences of *Aceria* from *Tamarix* from GenBank, including those from Africa (OR133633–OR133636) and India (KM114210), form a highly supported clade in the *Cox1* tree (Figure 10B). No one analysis recovered a distinct geographical pattern in the Tam-clade, viz., subclades corresponding to different continents (America/Africa/Europe/Asia). The results of both single gene analyses coincided in that the genus *Echinacrus* did not cluster with *Dicruvasates* within Tam-clade but belonged to non-related clades comprising sequences of various Anthocoptinae and Aceriini (Figure 10).

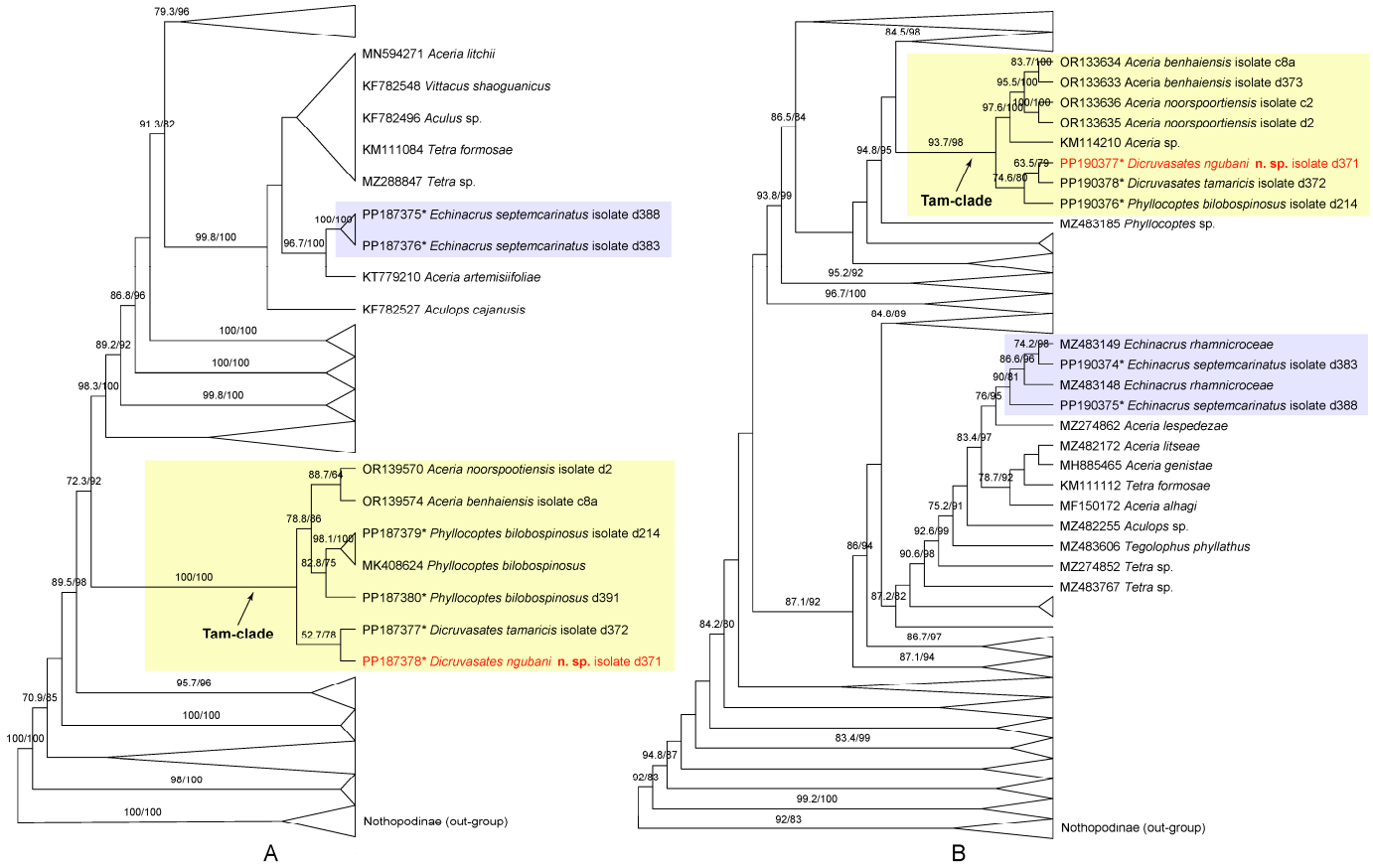


Figure 10. D1D2 28S (**A**) and Cox1 (**B**) maximum likelihood trees showing the relative position of *Tamarix*-associated eriophyids (colored yellow, Tam-clade) and *Echinacrus* (colored violet) in Eriophyidae. Values of SH-aLRT support (%, >70)/UFBS (%, >85) are shown above branches; in the Tam-clade, all support values are shown. New sequences obtained in this study are indicated by asterisks (*); the new *Dicruvasates* species is colored red.

4. Discussion

Rediagnosis of *Dicruvasates* and its relationship with *Echinacrus* and *Phyllocoptes*. Up to now, the genus *Dicruvasates* was monotypic and included a single species from a native tamarisk, *T. nilotica*, distributed in Arabia and the northern–eastern part of Africa. In this paper, we described a new species of this genus associated with the endemic southern African tamarisk, *T. usneoides*. The morphological comparison showed that these two *Dicruvasates* species, living on congeneric hosts in remote areas of Africa, differ only in a few characters. While qualitative characters separating them were relatively easy to determine, only statistical analysis allowed us to find the best morphometrics for discriminating these species. Previous studies showed cluster, discriminant, and canonical analyses, Random Forest, and geometric morphometrics powerful methods for separating morphologically similar populations of gall mites [40–45]. In this study, stepwise general discriminant analysis improved the differential diagnosis of *D. ngubani* **n. sp.** and determined the two best diagnostic morhometrics—lengths of leg setae *ft*″ II and *ft*′ II.

We also showed that the original diagnosis of the genus *Dicruvasates* was based on an inadequate interpretation of the morphology of the type species. Reinvestigation of the specimens of *D. tamaricis* from Egypt revealed that the two rod-like structures described as paired external horn-like processes preceding the frontal lobe are internal structures, palpal apodemes, which are typical for many eriophyoid taxa. The genera *Neoprothix* Reis and Navia, 2014 (Phytoptidae) and *Bucculacus* Boczek, 1961 (Diptilomiopidae) were two other cases when the erroneous interpretation of prosomal structures caused taxonomic problems in Eriophyoidea. The genus *Neoprothrix* was assigned to the problematic phy-

toptid subfamily Prothricinae Amrine, 1996 based on the presence of tiny, paired setae *vi* near the anterior margin of the prodorsal shield [46], but type species reinvestigation under CLSM showed that they are two tiny apodemes [47]. The genus *Bucculacus* was described as having an unusually long frontal lobe, depicted as an irregularly bent protuberance of the anterior part of the prodorsal shield in the original description [48], while later, the author of the genus admitted, and the investigation of the paratypes confirmed, that this elongated structure does not belong to the prodorsal shield but represents a deformed stylet sheath [49].

Genus *Dicruvasates* shares two remarkable structures with *Phyllocoptes bilobospinosus*: the supracapitular plate and the ventral telosomal ridge. The first structure has been previously reported in non-related vagrant eriophyids and some phytoptids. Along with variously shaped frontal lobes of Eriophyoidea, this plate presumably participates in protecting the cheliceral bases dorsally and contributes to the tightness of the feeding channel [50]. Presence of supracapitular plate supports the close relation of vagrant phyllocoptines from tamarisks found in our molecular analyses. The function of the ventral telosomal ridge is unknown; however, it may be associated with the anal secretory apparatus that is well-developed in these mites.

Dicruvasates spp. have small plates and/or spine-like processes on their dorsal opisthosomal annuli, forming distinct subparallel rows. Formation of similarly distributed variously shaped serial plates and processes on dorsal and lateral opisthosoma is known in many eriophyid genera of the subfamilies Phyllocoptinae, Tegonotinae, and Anthocoptinae, in some diptilomiopids, and a few vagrant phytoptids and nalepellids, but atypical for eriophyid subfamilies Nothopodinae, Cecidophyinae, and Eriophyinae [23,24,51]. Usually, such serial structures mark longitudinal opisthosomal ridges formed by uniformly curved dorsal annuli and are associated with wax production [52]. Here, we investigated the relationship between *Dicruvasates* and *Echinacrus* and showed that they belong to non-sister lineages of Eriophyidae comprising either taxa with serial opisthosomal plates/processes on ridges or without them. This observation suggests that the presence of serial plates/processes on opisthosomal ridges is one of the homoplastic traits, numerous in Eriophyoidea [5], which should be treated cautiously when grouping eriophyoid taxa and reconstructing the cladogenesis of Eriophyoidea.

The formation of longitudinal ridges is probably also homoplastic in Eriophyoidea. The ridges strengthen the dorsal cuticle and consolidate the mite body, which may be especially important for vagrant species [53]. Topographically longitudinal opisthosomal ridges correspond to the organization of subcuticular opisthosomal musculature, which is highly conservative in Eriophyoidea [54,55] and either form spaces (or separations) containing (or isolating) rows of serial elongated subcuticular muscles. Therefore, the ancestral type of opisthosomal musculature organization may be the prerequisite for the evolutionary transformation of the opisthosomal topographies that occurred similarly in non-related lineages of Eriophyoidea.

Transcontinental clade of gall mites from *Tamarix*. A few previous studies on the phylogeny of Eriophyoidea based on analyses of nuclear rDNA and mitochondrial genes showed the following: (a) Closely related plant taxa often host closely related eriophyoid taxa; (b) Taxonomically heterogeneous complexes of gall mites associated with one host plant genus or family may be monophyletic; (c) The evolution of eriophyoid mites does not precisely follow the evolution of their hosts in terms of cophylogeny; (d) However, in the evolution of some supraspecific mite taxa codivergency with large supraspecific host groups has happened [1–6]. Inferring the monophyly of the transcontinental clade *Tam*, containing species of the genera *Aceria*, *Phyllocoptes*, and *Dicruvasates* associated with tamarisks, is the most unexpected and interesting finding of this study. The clade *Tam* was recovered in all our analyses based on sequences of two genes (28S and Cox1), independently evolving in different cellular compartments (nucleus and mitochondria). These genes are known to be suboptimal for resolving deep phylogenetic relationships of Acariformes and Eriophyoidea because of hypervariable regions of 28S that are hard to align correctly and saturated

phylogenetic signal of the third triplet positions in Cox1 [56,57]. However, the clade *Tam* is biologically justified because it unites taxa associated with one plant genus, and morphologically, its members are generally very similar: they retain a complete set of all typical setae and differ only in putatively homoplastic traits of opisthosomal topography.

If the *Tam* clade truly exists, it would be interesting to test whether the *Tamarix*-associated species of the genus *Eriophyes*, which were not included in our analyses, also belong to this clade. One more question concerns the geographical distributions of *Dicruvasates* and *Phyllocoptes* from *Tamarix*. Current data show longitude distribution for *Dicruvasates* and latitude distribution for *Phyllocoptes* from tamarisks (Figure 1). If further field surveys confirm these geographical patterns and new species of *Dicruvasates* and *Phyllocoptes* will be found on tamarisks, it would be interesting to test if "suborthogonal" distributions of *Dicruvasates* and *Phyllocoptes* from *Tamarix* coincide with cladogenesis in *Tam*-clade and distributions of *Tamarix* spp.

Remarkably, the only *Tamarix*-associated *Aceria* from Asia that is present in GenBank is clustered with *Aceria* from South Africa in our molecular phylogenetic analyses. This clustering indicates evident transcontinental links of the eriophyoid faunas from geographically remote regions, which was also hypothesized before in the studies on eriophyoids from endemic African palms, conifers, and ferns [20,53,58,59]. These examples indicate still poorly investigated dispersal routes of gall mites and the history of their symbiosis with plants, including possible transcontinental and transoceanic dispersal events, long-term coexistence with host plant groups, host-shifts, and recent introductions [60–62].

Anal secretory apparatus (ASA) is present in immatures of gall mites. For the first time, with the aid of confocal microscopy, we demonstrated that immatures of *Dicruvasates* have elements of the ASA. This complex of internal structures, associated with the rectum and occupying the space of telosoma, is hypothesized to be a synapomorphy of Eriophyoidea evolved for silk production [20,54]. The ASA is recorded in all major lineages of Eriophyoidea, suggesting that most eriophyoids are capable of silk production. However, only a few taxa (e.g., aberoptines), due to their hypertrophied ASAs, produce a large enough amount of silk for creating protective web nests [20,52,63].

There is an open question: what is the function of the ASA in the majority of eriophyoids that do not produce nests? Anal glands, the main element of the ASA detected in previous transmission electron microscopy studies, were hypothesized to produce "drag lines" that could be impregnated with pheromones [20,54]. In the context of our discovery of the ASA in immatures of *Dicruvasates*, this hypothesis highly corresponds with observations by many authors in different countries on the behavior of males of gall mites in the presence of nymphs: several males often surround a quiescent female nymph and encircle her with spermatophores ([64]; J.A. and P.C unpublished observations). This behavior indicates that males can detect nymphs, probably due to the drag lines produced by the nymphal ASA. If this can be confirmed experimentally in the future, the more general hypothesis would be interesting to test: is the secret of the ASA the main component of non-contact signaling between gall mite individuals?

Contrary to other web-producing arthropods, current data on the chemical composition of the secretions of eriophyoid anal glands are very limited. A single study reports that the silk of the web-producing species *Aculops knorrii* contains proteins [63], while the silk genes of gall mites and the physical properties of their silk are completely unknown. Overall, the molecular biology of gall mite silk is a new unexplored field for future research that may have potential for developing new methods for controlling mites based on knowledge of their ecology, behavior, and silk gene genetics.

5. Conclusions

Revealing coevolutionary patterns in the parasitic system "gall mites—higher vascular plants" is a largely underexplored topic in eriophyoid studies. Large-scale comparative studies of host associations and geographical distributions, as well as comprehensive molecular phylogenetic analyses of supraspecific taxa of gall mites, are still very rare. The

molecular analyses of taxonomic complexes of gall mites associated with monophyletic groups of host plants is a promising approach for investigating mechanisms of cladogenesis in Eriophyoidea. In this study, we found a new molecular host-specific transcontinental clade of gall mites associated with the plant genus *Tamarix*. Along with the species of the genera *Aceria* and *Phyllocoptes*, which are non-monophyletic according to our results, this clade contains representatives of the rarely encountered small genus *Dicruvasates*, which is an example of an inadequately diagnosed eriophyid taxon. Erroneous interpretations of gall mite morphologies in old taxonomical literature are often correlated with low-quality optics that were available to previous authors. Additionally, the extreme miniaturization of eriophyoid mites makes them one of the most difficult groups of phytophagous arthropods for zoologists. It is not surprising that some of their internal structures, e.g., genital tubules, spermathecal process, and even complexes of structures like silk-producing anal secretory apparatus (ASA), were discovered only recently. In the era of fast-developing new research technologies, eriophyoid mites await investigations using advanced molecular and bioinformatics approaches as well as more classic microscopy techniques, e.g., X-ray microtomography, immunohistochemistry, and confocal and electron microscopy, which were already applied to various larger arthropods. Finally, molecular biology studies of gall mite silk and comparative studies of the anatomy of the ASA may be promising directions for the future research of Eriophyoidea.

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