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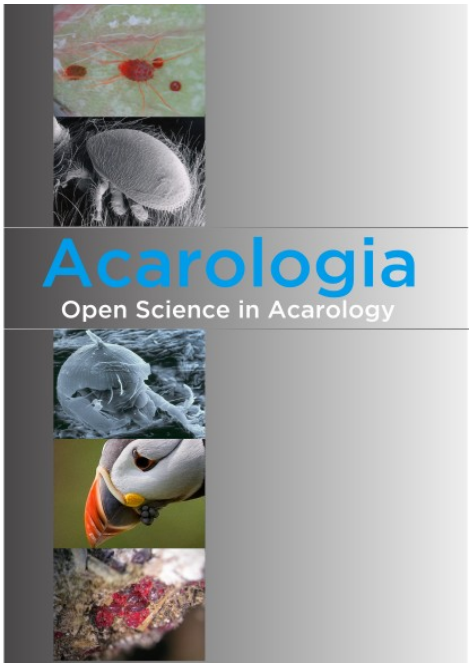

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Two new alien *Eutetranychus* mites (Prostigmata, Tetranychidae) for Madeira Island, Portugal

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Original research

ABSTRACT

Two field surveys on agricultural and non-agricultural plants in Madeira Island, and examination of slides of the Laboratório de Qualidade Agrícola (LQA, Madeira) collection, disclosed two species belonging to the genus *Eutetranychus*: the Texas citrus mite *Eutetranychus banksi*, associated with *Citrus x aurantium*, *Citrus limon*, *Citrus reticulata*, *Phaseolus vulgaris* (new host record), *Plumeria rubra* and *Ricinus communis*, and the African red mite *Eutetranychus africanus* on *Citrus medica* (new host record), *Carica papaya*, *Vitis vinifera* and *R. communis*. Both species are new records for Madeira Island, and this is the first record of *E. africanus* for a European country. Molecular analysis (18S rRNA gene) of *E. africanus* confirmed morphological identification. This integrative approach of comparing morphological and molecular characters with validated references allowed us to reliably identify this species, which has repeatedly been misidentified in the past.

Keywords spider mite; integrative taxonomy; African red mite; Texas citrus mite

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Introduction

Alien arthropods jeopardize food supplies, endanger valued species, cause economic losses, disrupt ecosystem functions and threaten human health, with the accelerating pace of commercial and social globalization creating unprecedented opportunities for the movement and introduction of species to new areas of the world (Venette and Hutchison, 2021). Climate change can also create favourable conditions for pest movement, invasion, and establishment worldwide, as it can affect plant health and facilitate the introduction and installation of unwanted organisms (Gullino *et al.* 2022).

In the last decades, several species of allochthonous (non-native) spider mites of the family Tetranychidae have been introduced into Europe (Navajas *et al.* 2010). Species occasionally causing major damage to crops in Europe include *Tetranychus evansi* Baker & Pritchard on solanaceous crops, and *Oligonychus perseae* Tuttle, Baker & Abbatiello on avocado (for reviews see Boubou *et al.* 2011 and Bragard *et al.* 2022, respectively), while *Eutetranychus*

spp. mites are important pests of citrus in southern mainland Portugal (Naves *et al.* 2021) and Spain (Ferragut *et al.* 2013). Moreover, new introductions continue to occur, resulting in additional alien species being established in Europe (e.g., Naves *et al.* 2021).

In Madeira Island eight species of spider mites have been reported until now, including alien and ubiquitous species with unknown status such as *Eotetranychus lewisi* (McGregor), *O. perseae*, *T. evansi*, *Tetranychus ludeni* Zacher and *Tetranychus urticae* Koch (Naves *et al.* 2021). Geologically, Madeira is located in the northwest sector of the African Tectonic plate (Brum da Silveira *et al.* 2012), being a political European territory and a Portuguese Autonomous Region. In this note we report the presence of two additional alien mites for Madeira, the Texas citrus mite *Eutetranychus banksi* (McGregor), and the African red mite *Eutetranychus africanus* (Tucker), this being the first report of *E. africanus* for a European country.

Material and methods

Mite collection

Collection of mites from plant material was undertaken in Madeira Island from May to October 2022. Mite sampling was performed in 50 localities, comprising 44 species (including agricultural and non-agricultural plants) of 23 plant families. Adult mites of both sexes were collected directly from leaves with a brush under a stereomicroscope or following the dipping washing method (Boller 1984), gathering mites on sieves of various sizes for observation under a stereomicroscope. Specimens were kept in 100% ethanol (molecular study) and 96% ethanol (morphological study).

Morphological analyses

Mites were cleared in lactic acid (50%) for a day and mounted in Hoyer's medium for phase contrast microscope observation.

Slides of the Laboratório de Qualidade Agrícola (LQA, Madeira) collection, containing *Eutetranychus* collected during 2020-2023, were also examined.

Morphological identifications were performed using the descriptions and keys of Meyer (1974, 1987) and Kamran *et al.* (2018) for the genus *Eutetranychus*.

Molecular analyses

For *E. africanus*, an integrative approach combining morphological and molecular data was used.

Total DNA from three individual mites (collected on *Ricinus communis* L. in Funchal, Madeira; Table 1) was extracted separately using the DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) following the protocol provided by the manufacturer. The isolated DNA was directly used as a template for PCR amplification of the 18S ribosomal RNA gene using two primer sets: 1096F (5′GGTAATTCTGGAGCTAATAC3′) and 1912R (5′TTTACGGTCAGAACTAGGG3′) (Holterman *et al.* 2006); 18S-2F (5′TGGCCTCTGAGCCGACGATGTAT3′) and 18S-8R (5′TCTCGTTCGTTATCGGAATTAAC3′) (Matsuda *et al.* 2014). For a final volume of 25 µL, the PCR reaction mixture consisted of 12.5 µL of Platinum II HotStart PCR Master Mix (2X) (Thermo Fisher Scientific, Lithuania), 0.75 µL of each primer (10 µM), and 1 µL template DNA.

The PCR conditions were: (i) initial denaturation at 95 °C for 3 min; (ii) 5 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, and extension at 68 °C for 30 s; followed by (iii) 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 68 °C for 30 s; and (iv) final extension at 68 °C for 5 min. PCR products were cleaned using EXO-SAP (Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase,

ThermoScientific, CA, USA) following the manufacturer's recommendations. The amplified PCR products were sequenced at the Sequencing Facility at INIAV with the same primers used for their amplification. Multiple sequence alignment was carried out using the Clustal-W program of BioEdit (Hall, 2007) and revealed no heterogeneity in the nucleotide composition of the 18S sequences for the three individuals. Thus, representative consensus sequences made from the forward and reverse sequences (999 bp and 1067 bp) were BLAST against the NCBI GenBank database to retrieve the closest accessions.

Results and Discussion

Two alien species belonging to the genus *Eutetranychus* were detected in Madeira: *E. banksi* and *E. africanus*. While *E. banksi* is already known from Portugal and Spain, the nearest country with the presence of *E. africanus* is Egypt (Attiah, 1967). The updated distribution map of both species is presented in Figure 1.

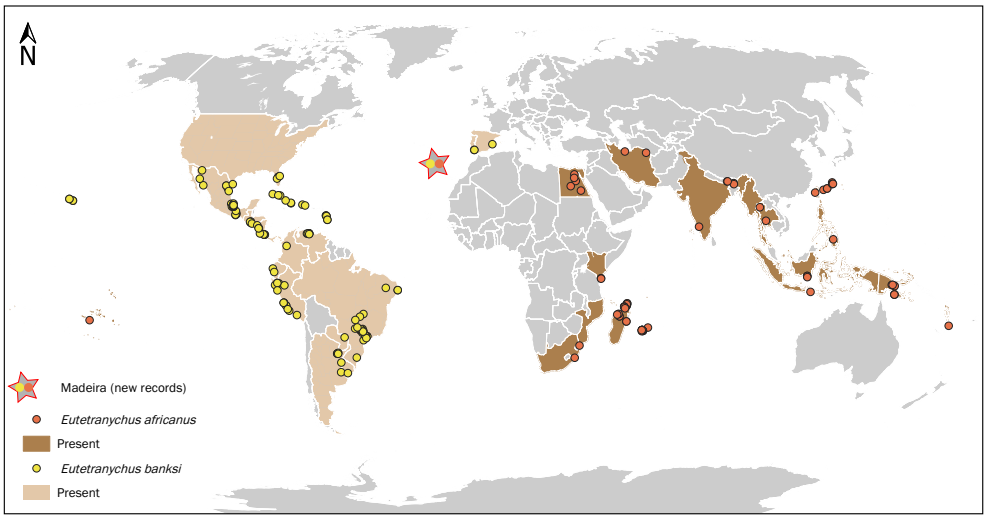

Figure 1 World distribution map of *Eutetranychus africanus* and *Eutetranychus banksi*. For each species, country level (data derived from Migeon and Dorkeld, 2023) and geo–localized records are indicated.

Family Tetranychidae Donnadieu, 1875

Tribe Eurytetranychini Reck, 1950

Genus *Eutetranychus* Banks, 1917

Neotetranychus (*Eutetranychus*) Banks, 1917: 177.
Type species: *Tetranychus banksi* McGregor, 1914: 358

Eutetranychus banksi (McGregor, 1914)

The Texas citrus mite is reported from Afrotropical, Australasian, Nearctic, Neotropical, and Palearctic regions (Migeon and Dorkeld 2023). In Europe it is found in southern Portugal and Spain (Ferragut *et al.* 2013) (Figure 1), being one of the most important pests affecting citrus partly because of limited biological control by natural enemies and disruptions following insecticide applications (López-Olmos and Ferragut 2023).

Records: in Madeira, *E. banksi* was collected from several locations and in six host plants, three of which of the genus *Citrus* (Table 1). One of the plant hosts was *Phaseolus vulgaris*

(Fabaceae), the common bean, which is a new host to this species (Migeon and Dorkeld 2023). The discovery of specimens collected in 2020 within the LQA collection implies that *E. banksi* is present in the Island for, at least, three years. Sampled plants had no significant damages visible.

Distinctive characters: females of this species are separated of closely species of the *banksi*-group (16 species, see Kamran *et al.* 2018) by having coxa II with 2 setae, members of setae e_1 and f_1 forming a trapezoid pattern, genua I and II bearing 4 setae, tibia III bearing 4–5 setae and tibia IV 5–6 setae (Figure 2).

Note: new species for Madeira Island; new host record on *Phaseolus vulgaris*.

Eutetranychus africanus (Tucker, 1926)

The African red mite is reported from tropical and subtropical countries of the Afrotropical, Australasian and Oriental regions, and also in Iran and Egypt in the Palearctic region (Migeon and Dorkeld 2023). Damage to citrus and papaya has been reported in Africa (e.g. Kiptoo *et al.* 2022) and Asia (e.g. Ho *et al.* 2015).

Records: in Madeira, *E. africanus* was collected in four locations and four host plants (Table 1). Mites were found mainly on the upper surface of the leaves, particularly near the mid-rib, and feeding damage was observed (Figure 3).

Distinctive characters: females of this species are separated of other species of the *banksi*-group (16 species, see Kamran *et al.* 2018) by having coxa II with 2 setae, setae f_1 members equally spaced or slightly more widely spaced than setae e_1 members, dorsal striation between members of setae d_1 and e_1 forming a "V-pattern", dorsocentral setae c_1, d_1 and e_1 inserted on obvious/prominent tubercles, 6 setae on tibiae II and III, 2 or 3 setae on genu III, 2 setae on genua II and IV (Figure 4).

The molecular analysis confirmed the morphological identification, with a positive identification consisting of a BLAST hit with 100% identity of an alignment length of 999 nucleotides with a reference database sequence, belonging to *E. africanus* (accession number AB926232) from Japan (Matsuda *et al.* 2014). The novel sequences obtained in this study were deposited in GenBank under the accession numbers OQ312050 and OQ411006. As misidentification of *E. africanus* as *E. orientalis* has occurred repeatedly (e.g., Ehara and Gotoh 2007), despite the two species being easily separated using the coxa II setal count (2 setae present in *E. africanus* vs. 1 seta in *E. orientalis*), our integrative approach, comparing both morphological and molecular characters to validated references, emphasizes its value in obtaining reliable identification of these species.

Table 1 Species of the genus *Eutetranychus* collected in Madeira Island and respective host plants. Mites marked with [1] refer to the LQA acarological collection (Madeira). [2] indicates the population subjected to molecular analyses.

Species	Host	Coordinates	Location (Parish)	Altitude (m a.s.l.)	Date of collection
Eutetranychus africanus	*Citrus medica*	32.6617°N -16.9806°W	Câmara de Lobos	233	17 May 2022
Eutetranychus africanus [1]	*Carica papaya*	32.6795°N -17.0744°W	Ribeira Brava	117	31 May 2022
Eutetranychus africanus	*Vitis vinifera*	32.6599°N -16.9747°W	Câmara de Lobos	196	12 Oct. 2022
Eutetranychus africanus [2]	*Ricinus communis*	32.6573°N -16.9003°W	Funchal	28	13 Oct. 2022
Eutetranychus banksi [1]	*Citrus x auratium*	32.6548°N -16.8331°W	Santa Cruz	261	07 Sept. 2020
Eutetranychus banksi [1]	*Plumeria rubra*	32.6435°N -16.8370°W	Santa Cruz	86	30 Aug. 2022
Eutetranychus banksi	*Ricinus communis*	32.6731°N -16.8101°W	Santa Cruz	225	13 Oct. 2022
Eutetranychus banksi	*Ricinus communis*	32.6527°N -16.8179°W	Caniço	76	13 Oct. 2022
Eutetranychus banksi [1]	*Phaseolus vulgaris*	32.6589°N -16.9112°W	Funchal	139	16 May 2023
Eutetranychus banksi [1]	*Citrus reticulata*	32.7244°N -17.1793°W	Calheta	165	30 June 2023
Eutetranychus banksi [1]	*Citrus limon*	32.6934°N -17.1096°W	Ponta do Sol	338	10 July 2023

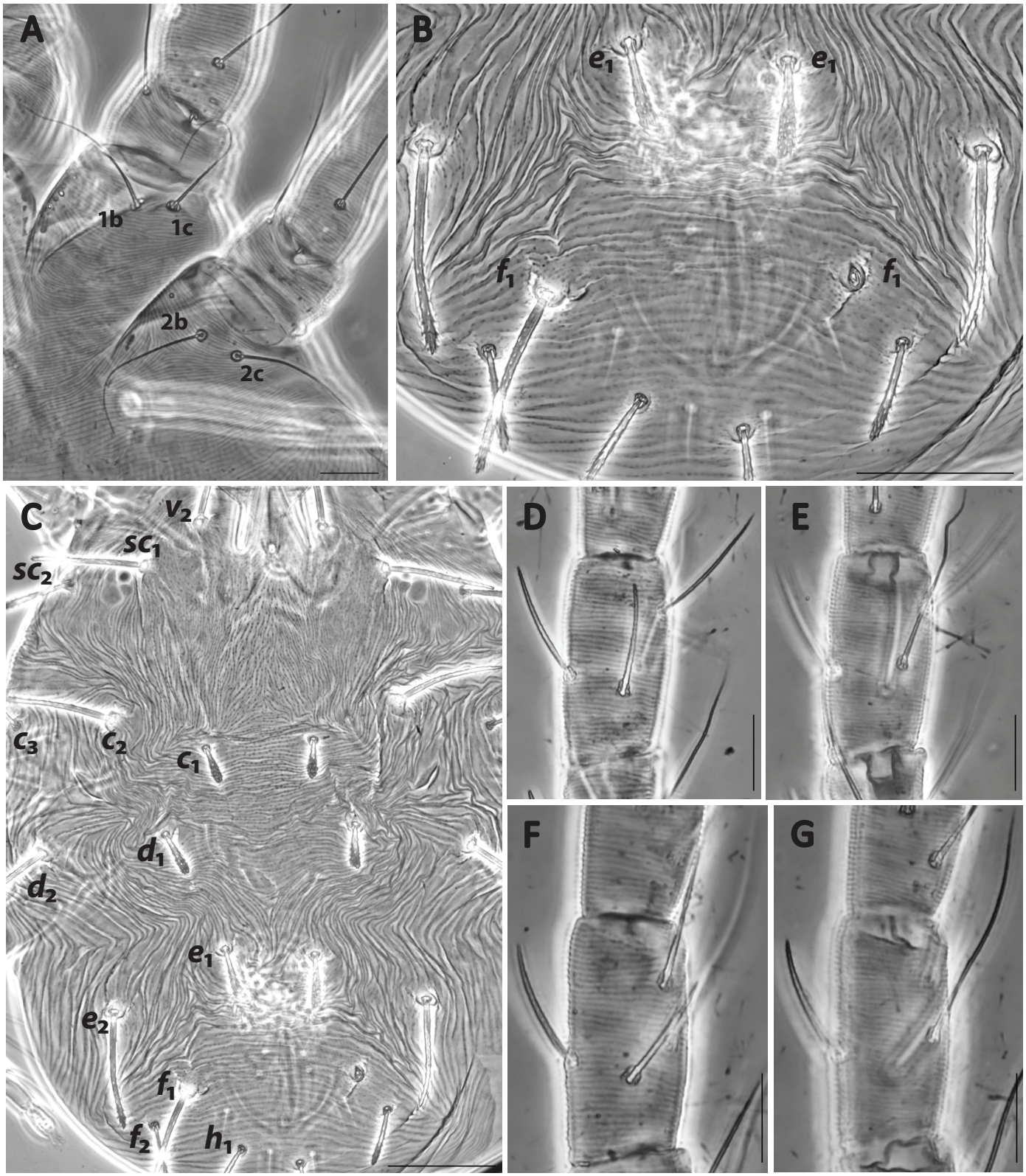

Figure 2 *Eutetranychus banksi* female: A – two setae present on coxae I and II; B – members of setae e_1 and f_1 forming a trapezoid pattern; C – dorsal setae set on small tubercles; D–E – 4 setae on genu I: 3 dorsal (D) and 1 ventral (E); F–G – 4 setae on genu II: 3 dorsal (F) and 1 ventral (G). Scale bars A, D–G=20μm, B–C=50μm.

Figure 3 Feeding damage by *Eutetranychus africanus* on *Citrus medica* leaf, Madeira Island.

Note: new species for Madeira Island and second record for the west Palearctic region; new host record on *Citrus medica*.

There is no information on how the two alien *Eutetranychus* arrived in Madeira or how long they have been present, but the movement or trade of mite-infested plant material is the most likely hypothesis to explain their introduction. The climate of southern Madeira can certainly provide suitable climatic conditions for their persistence, considering the mean annual temperature of 18 °C (Mora and Vieira 2020).

Although *E. africanus* is mainly found in regions with tropical or sub-tropical climates (Figure 1), its presence in northern Iran suggests the ability to survive cold winters, and therefore this species may represent an additional challenge for citrus orchards of the Mediterranean basin. Precautionary measures should be applied to contain the populations in Madeira and prevent future disseminations of this alien mite, considering the damages reported on citrus, papaya, and on other crops worldwide. We also recommend that surveys should be conducted in continental Europe to check the presence of *E. africanus*. It is possible that this species occurs in mixed infestation with other *Eutetranychus* species and has been unnoticed until now.

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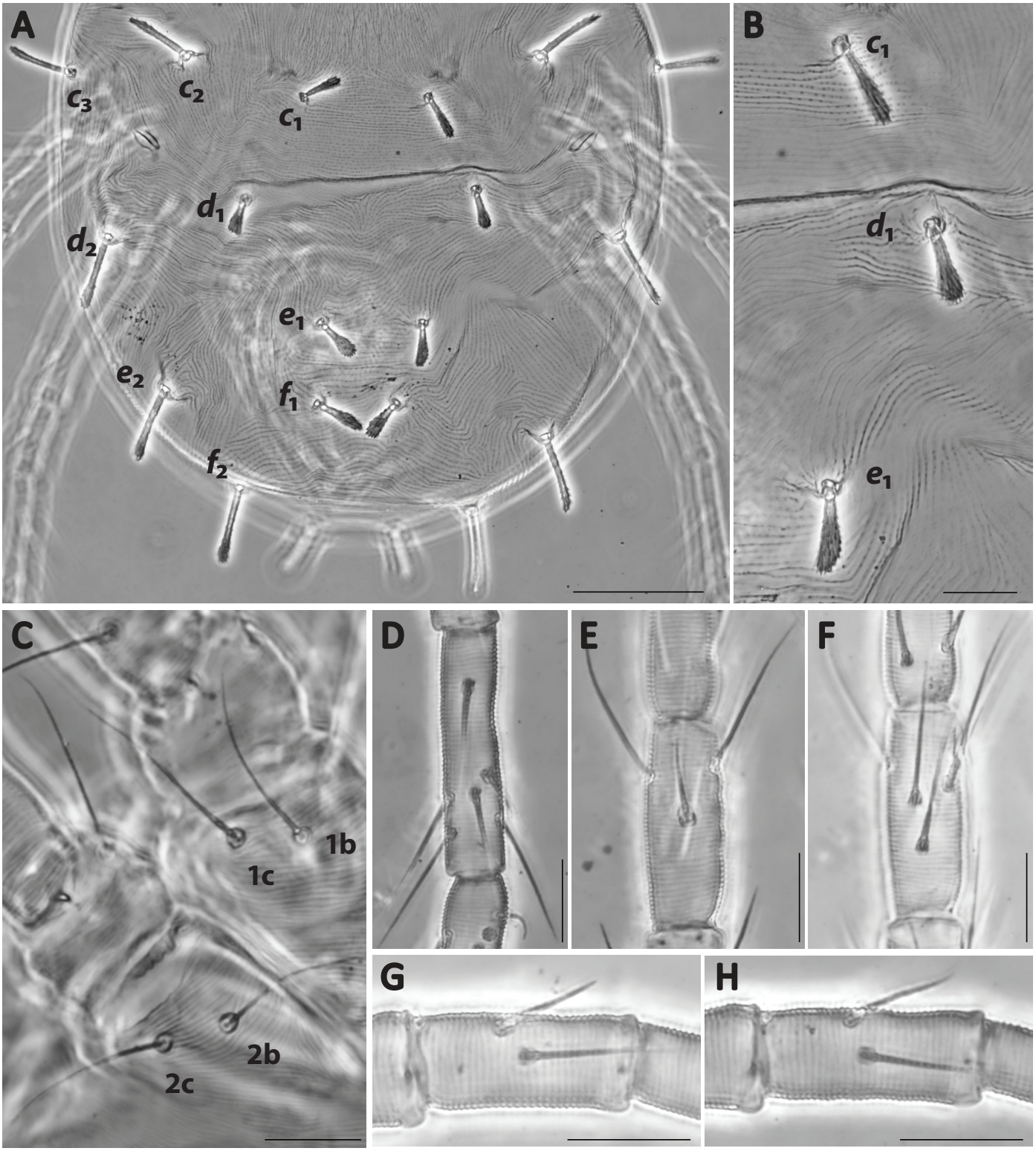

Figure 4 *Eutetranychus africanus* female: A – dorsal opisthosoma with : setae f_1 in normal position, striation forming a "V-pattern" between members of setae d_1 and e_1; B – first three pairs of dorsocentral setae (c_1, d_1 and e_1) inserted on obvious tubercles; C – two setae present on coxae I and II; D – 6 setae on tibia III; E–F – 6 setae on tibia II: 3 dorsal (E) and 3 ventral (F); G – two setae on genu II; H – two setae on genu IV. Scale bars A=50μm, B–H=20μm.

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