

Three new species of the genus *Pterygosoma* Peters, 1849 (Acariformes: Pterygosomatidae) from agamid lizards (Sauria: Agaminae) with DNA barcode data

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Abstract Three new species of the genus *Pterygosoma* Peters, 1849 parasitising lizards of the subfamily Agaminae (Squamata: Agamidae) are described: *P. pallidum* n. sp. from *Trapelus pallidus* (Merrem) and *P. parasiniatum* n. sp. from *Pseudotrapelus* cf. *sinaitus* (Heyden) (both from Jordan); and *P. theobaldi* n. sp. from *Phrynocephalus theobaldi* Blyth from North India. We extend the standard morphological description of the new species by using DNA barcode markers, partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (*cox1*) gene and the hypervariable region D2 of the nuclear 28S rRNA gene. A key to the species group *inermis* is constructed based on female morphology. The agamid genus *Phrynocephalus* Kaup, 1825 is recorded as a host of *Pterygosoma* for the first time.

Introduction

Most mites of the family Pterygosomatidae Oudemans, 1910 (Acariformes: Prostigmata) are permanent and highly specific (mono- or stenoxeous) ectoparasites of lizards (Squamata: Sauria), with the exception of *Geckobia enigmatica* Bertrand & Pedrono, 1999, found on tortoises (Testudines: Testudinidae) (Bertrand & Pedrono, 1999), and species of the genus *Pimeliaphilus* Trägårdh, 1905, found on terrestrial arthropods (Paredes-León et al., 2012).

To date, the family Pterygosomatidae includes 179 species grouped into nine genera recorded from all zoogeographical regions, except for the Antarctic (Fajfer, 2012).

Pterygosoma Peters, 1849 is one of the most diverse and species-rich pterygosomatid genera with 67 species grouped into two subgenera: *Pterygosoma* (*sensu stricto*) with 64 species associated with the South American tree lizards (Liolaemidae) and with the African and Asian agamas (Agamidae); and *Gerrhosauropia* with three species from the African plated lizards (Gerrhosauridae) (Fajfer, 2016).

In the last few years several studies dedicated to the systematics of species of *Pterygosoma* have been published (Bochkov et al., 2009; Fajfer & González-Acuña, 2013; Fajfer & Melnikov, 2014). All species descriptions made so far had been based exclusively on external morphology. This was mostly dictated by the fact that the studies were carried out on mites

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collected from museum host specimens captured at the turn of the 20th Century and initially preserved in industrial methylated spirit. In general, in such material DNA is too degraded to undertake the molecular-based investigations.

Recently, the DNA barcodes based on the mitochondrial cytochrome *c* oxidase subunit I (*cox1*) supplemented with the D1–D2 hypervariable region of the nuclear 28S rRNA gene have been used as a practical tool in taxonomic studies of animal species (Hebert et al., 2003; Sonnenberg et al., 2007) including acariform mites (Dabert et al., 2008; Głowska et al., 2012; Mironov et al., 2012). As a result, the thorough external morphological descriptions accompanied by DNA barcodes helped to resolve many taxonomical problems, e.g. detection of cryptic species (Martin et al., 2010; Skoracka et al., 2012) or validation of the species status within populations of mites from various hosts (Głowska et al., 2013; Dabert et al., 2015).

In this paper we describe three new species of *Pterygosoma* associated with agamid lizards from the Palaearctic region and extend the standard morphological descriptions with DNA barcode data for the *cox1* and 28S rRNA gene fragments obtained from the type-material of *P. parasinatum* n. sp. from *Pseudotrapelus* cf. *sinaitus* (Heyden); *P. theobaldi* n. sp. from *Phrynocephalus theobaldi* Blyth; and *Pterygosoma pallidum* n. sp. from *Trapelus pallidus* Reuss. The two former species, according to the pattern of body setation, together with *P. inermis* Trägårdh, 1905, *P. inermis stoliczkana* Jack, 1962, *P. tuberculata* Jack, 1962, *P. adramitana* Jack, 1961, *P. sinaita* Jack, 1961, *P. aqabensis* Fajfer & Melnikov, 2014 and *P. dhofarensis* Fajfer & Melnikov, 2014, belong to the *inermis* species group established by Jack (1962a, b), whereas *P. pallidum* does not belong to any of the previously described groups within the genus. A key to the species of the *inermis* species group based on female morphology is constructed. Mites of the genus *Pterygosoma* are recorded on agamas of the genus *Phrynocephalus* Kaup for the first time.

Materials and methods

Morphological analysis

Mites used in the present study were kept in 70% ethanol and, before mounting in Hoyer's medium,

were cleared and softened in Nesbitt's solution at +45°C for 1–5 hours. Then, the mites were studied using the light microscope Olympus BH-2 with differential interference contrast (DIC) illumination and drawings were made using a camera lucida drawing attachment. All measurements are given in micrometres as the data for the holotype followed by the ranges for the paratypes. Nomenclature of the leg and idiosomal setae follow Grandjean (1939, 1944) and names of the palpal setae follow Grandjean (1946) as adapted to the family Pterygosomatidae by Bochkov & OConnor (2006). The scientific names of lizards follow Sindaco & Jeremčenko (2008) and Uetz & Hošek (2014). For detailed differential diagnoses of the new species, the type-specimens of *Pterygosoma mutabilis* Jack, 1961, *P. sinaita* Jack, 1961 and *P. inermis* (Trägårdh, 1905) deposited in the Natural History Museum, London, UK, were re-examined.

Molecular analysis

Mite specimens designated for DNA extraction were kept in 96% ethanol and, before mounting on microscope slides, three specimens of *Pterygosoma pallidum* n. sp., two specimens of *P. parasinatum* n. sp. and five specimens of *P. theobaldi* n. sp. were subjected to DNA extraction. Total genomic DNA was extracted from whole specimens using DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) as described by Dabert et al. (2008). Initially the *cox1* gene fragment (covering c.620 bp of the 5' terminus of *cox1* gene) was amplified by PCR using the primers bcdF01 (5'-CAT TTT CHA CTA AYC ATA ARG ATA TTG G-3') and bcdR04 (5'-TAT AAA CYT CDG GAT GNC CAA AAA A-3'). If necessary, the more specific forward primers Aseq01F (5'-GGA ACR ATA TAY TTT ATT TTT AGA-3') or Aseq04F (5'-GGA AAT TTA TAY TTY YTA TTT AGA AT-3') (Głowska et al., 2014) were used for *P. theobaldi*, and *P. pallidum* and *P. parasinatum*, respectively. The D2 region of the 28S rRNA gene was amplified using the primers 28F0001 (5'-ACC CVC YNA ATT TAA GCA TAT-3') and 28R0990 (5'-CCT TGG TCC GTG TTT CAA GAC-3') (Mironov et al., 2012).

PCR amplifications were carried out in 10 µl reaction volumes containing 2 µl of Hot FirePol Blend Master Mix (Solis Biodyne, Tartu, Estonia), 0.5 µl of each primer (10 µM) and 4 µl of DNA template using a thermocycling profile of 12 min at 95°C followed by

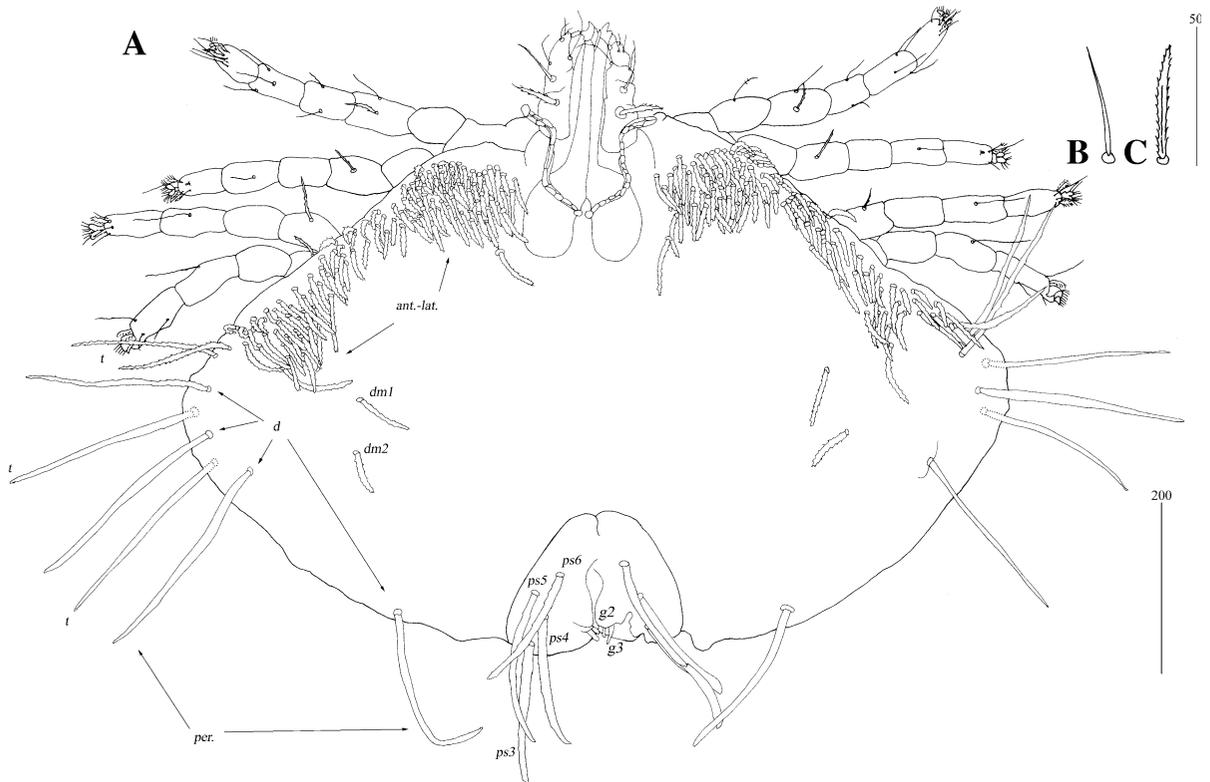


Fig. 1 *Pterygosoma pallidum* n. sp. Female. A, Dorsal view; B, Dorsal tibial seta of leg I (*dTiI*); C, Dorsal femoral seta of leg I (*dFI*). Setal notations: *d*, dorsal; *t*, terminal; *ant.-lat.*, antero-lateral; *per.*, peripheral

15 s at 95°C, 35 steps of 1 min at 50°C, 1 min at 72°C with a final step of 7 min at 72°C. After amplification, the PCR products were two-fold diluted with water and 5 µl of each sample was analysed by electrophoresis on a 1.0% agarose gel. Samples containing visible bands were purified with thermosensitive Exonuclease I and FastAP Alkaline Phosphatase (Fermentas, Thermo Scientific, Carlsbad, USA) and sequenced with BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems, Foster City, CA, USA) using the PCR primers. Sequence chromatograms were checked for accuracy and edited using Chromas Lite 2.1.1 (Technelysium Pty Ltd., Australia). Alignments of the sequence data were prepared manually with GenDoc v.2.7.000 (Nicholas & Nicholas, 1997).

Pairwise distances between nucleotide sequences were calculated using Kimura's 2-parameter (K2P) distance model (Kimura, 1980) for all codon positions with MEGA 5.05 software (Tamura et al., 2011).

The exoskeletons of the specimens sequenced and the corresponding DNA samples are

deposited in the collection of Adam Mickiewicz University. All sequences have been deposited in GenBank under accession numbers KT962103–KT962107.

Specimen depositories and reference numbers are cited using the following abbreviations: AMU, Adam Mickiewicz University, Department of Animal Morphology, Poznan, Poland; NHM, Natural History Museum, London, United Kingdom; ZISP, Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

Family Pterygosomatidae Oudemans, 1910

Genus *Pterygosoma* Peters, 1849

Subgenus *Pterygosoma* Lawrence, 1959 (*sensu stricto*)

Pterygosoma pallidum n. sp.

Type-host: *Trapelus pallidus* (Merrem) (Sauria: Agamidae).

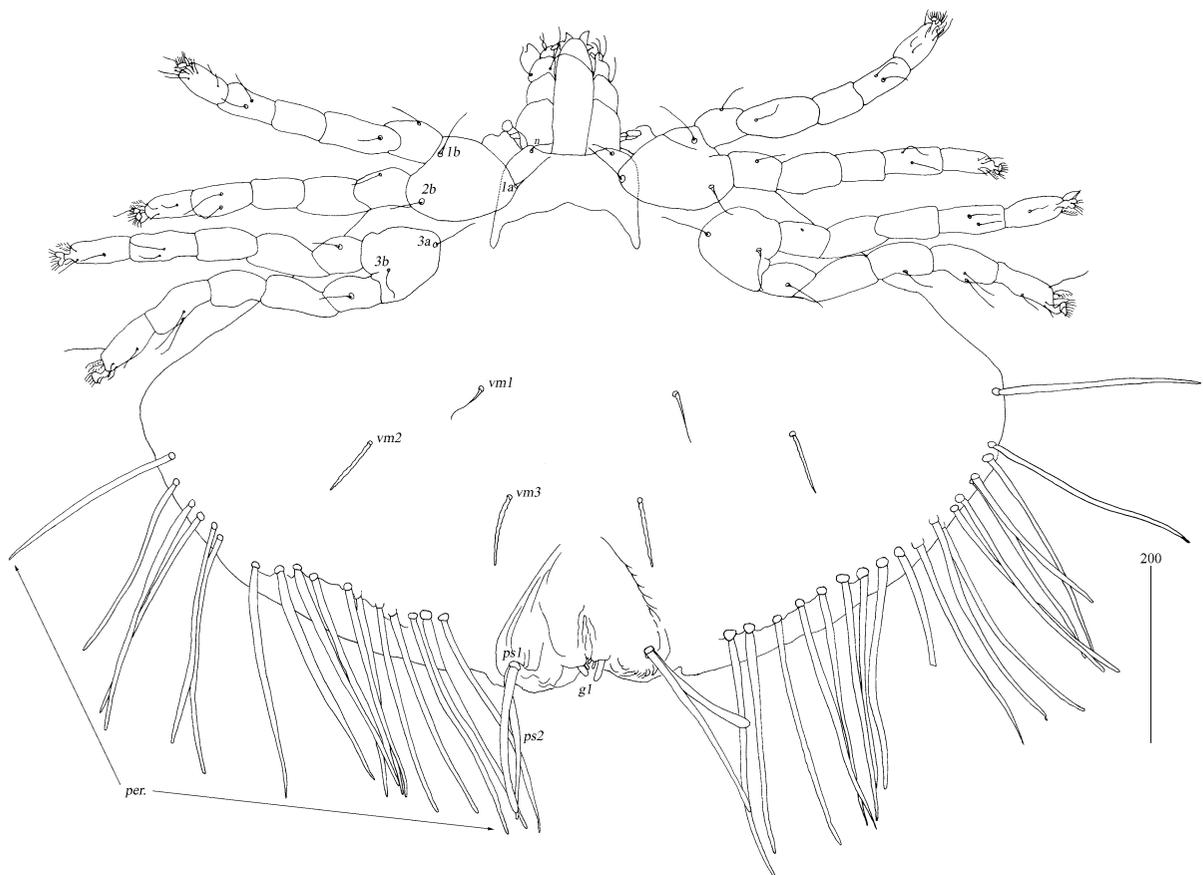


Fig. 2 *Pterygosoma pallidum* n. sp. Female, ventral view

Type-locality: Ma'an Province, 19 km north of Al-Jafr (ZISP 28779, 28782, 28784), Jordan; Aqaba Province, 22 km west of Ash-Sawbak (ZISP 28861); Aqaba Province, 11 km north-east of Ar-Risha (ZISP 28783); Az Zarqa' Province, 10 km south of Azraq (ZISP 28863); Mafraq Province, 9 km south-east of Al-Bishriyya (ZISP 28862) (2012, coll. by Daniel Melnikov), Jordan.

Type-material: Holotype female is deposited in the ZISP (ZISP Reg. No. AVB 15-0910-001). Paratypes: 12 females, 3 males, 5 deutonymphs, 7 protonymphs and 8 larvae; of these, 4 females, 1 male, 2 deutonymphs, 3 protonymphs and 3 larvae are deposited in the ZISP (ZISP Reg. No. AVB 15-0910-001) and 8 females, 2 males, 3 deutonymphs, 4 protonymphs and 5 larvae are deposited in the AMU (Reg. Nos. AMU-PTE11.1 and AMU-PTE11.2). All mites were collected by D. Melnikov.

Etymology: The specific epithet is derived from the host species name.

Representative DNA sequences: KT962104 (*cox1*); KT962105 (28S rDNA).

Description (Figs. 1–7)

Female [Based on the holotype and 12 paratypes, Figs. 1–3.] *Gnathosoma*. Chelicerae 265 (260–265) long, basal swollen part 105 (105–120) long, slender distal part 160 (135–160) long. Fixed cheliceral digit 25 (20–25) long, with several tines. Movable cheliceral digit with basal spur. Palpal femur with slightly serrate seta *dF* 55 (45–55) long; palpal genu with smooth seta *dG* 60 (50–60) long; palpal tibia with 3 smooth setae: *dTi*, *l'Ti*, *l''Ti* and short claw, *c.5* long; palpal tarsi with 3 smooth setae. Subcapitulum with pair of smooth setae *n*, 25 (20–25) long. Each branch of peritremes 165 (145–180) long. Hypostome 200 (185–205) long, with several apical teeth. *Idiosoma* 615 (545–800) long, 915 (815–1,170) wide. Antero-lateral part of dorsum with *c.120* pairs of serrate setae

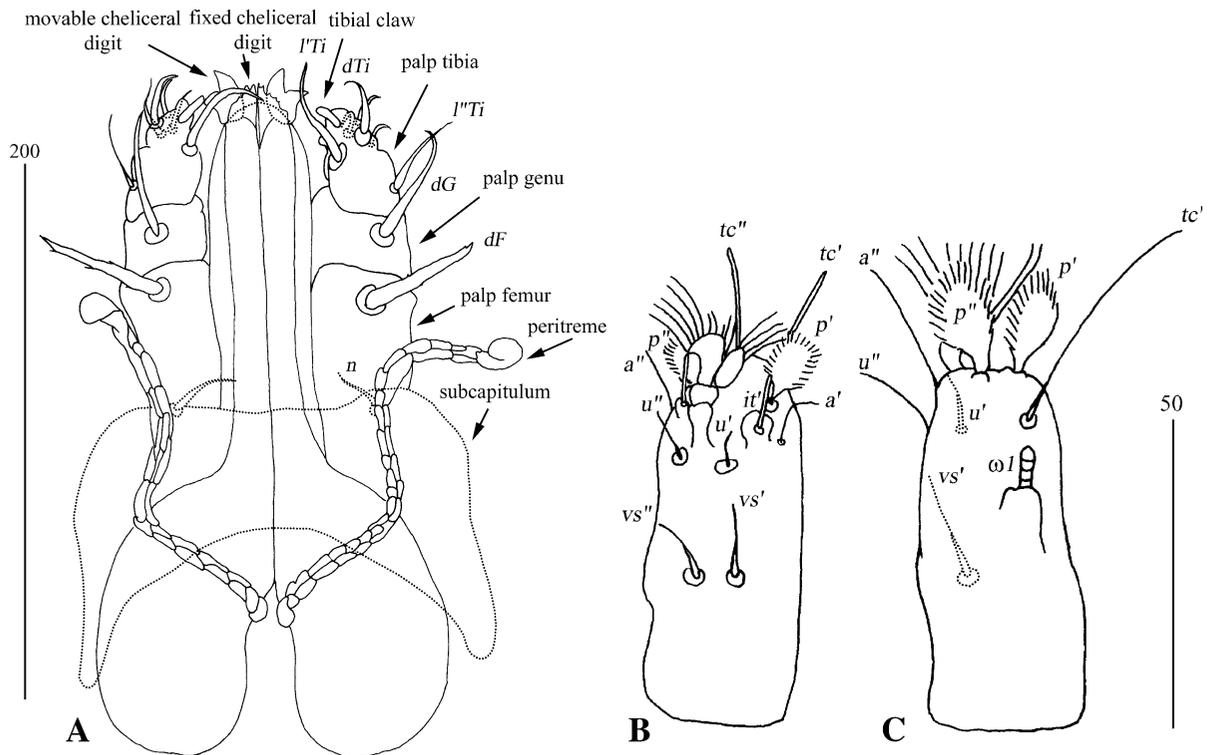


Fig. 3 *Pterygosoma pallidum* n. sp. Female, details. A, Gnathosoma, dorsal view; B, Tarsi I, ventral view; C, Tarsi II, dorsal view. Setal notations: *d*, dorsal; *l*, lateral. Podomere abbreviations: *F*, femur; *G*, genu; *Ti*, tibia

that increase in length from anterior (30–65 long) to posterior part (70–115 long); medial part with 2 pairs of dorso-median serrate setae *dm1* and *dm2*, 65 (50–65) and 55 (45–55) long, respectively. Posterior part with 24 and 26 pairs (22–34) of peripheral setae with barely discernible serration inserted on right and left side on idiosoma, 210–275 (160–265) long, inserted in 2 rows: dorsal row of 4 (4) pairs of setae (2 pairs near lateral part of idiosoma, 1 pair near genital slit and 1 pair between these), terminal row of 20 and 22 (18–30) pairs of setae on right and left side, respectively. Eyes present. Venter with 3 pairs of ventro-median setae *vm1*–*vm3*. Setae *vm1* filiform, *vm2* and *vm3* serrate. Genital slit situated terminally. Genital setal series represented by 3 pairs of slightly apically serrate setae *g1*–*g3*. Setae *g1* and *g2* inconspicuous, 15 (10–25) long, *g3* thick, 25 (25–45) long. Pseudanal setal series represented by 6 (4–6) pairs of setae with barely discernible serration in distal half, 140–180 long. *Legs*. Coxal setation: *1a*, *1b*, *2b*, *3a*, *3b* arranged in formula 2–1–2–0; all setae filiform. Setation of trochanters-tibiae corresponding to group

3 of Jack (1964): setae of trochanters I–IV (1–1–1–1), femora I–IV (3–1–1–1), genua I–IV (2–0–0–1), tibiae I–IV (5–3–3–3). In 5 paratypes additional reduced spine-like seta *dGI* (2 long) present. All setae on each podomere smooth, except for serrate setae *d'FI*–*IV*, *d'TiII*–*IV* and slightly serrate setae *l'GI*, *v'GII*, *v'TiIII*–*IV*, *v'TiIII*–*IV*. Setation of tarsi corresponding to group A of Jack (1964): I 13 setae (*ft*, *tc'*, *tc''*, *p'*, *p''*, *a'*, *a''*, *it'*, *it''*, *u'*, *u''*, *vs'*, *vs''*) and solenidion ωI ; II 8 setae (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*) and ωI ; III and IV with 8 setae each (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*). Setae *tc'*, *tc''*, *it'*, *it''* of leg I in form of eupathidia, *p'* and *p''* fan-like. Setae *vs'* and *vs''* of leg I, *tc'* of legs II and setae *a'*, *a''*, *u'*, *u''* of legs I–IV smooth, setae *tc'* of legs II–IV and setae *vs'* of legs III–IV slightly serrate.

Male [Based on 3 paratypes, Fig. 4.] *Gnathosoma* as in female. Chelicerae 90–100 long, basal swollen part 50–55 long, slender distal part 40–50 long. Fixed cheliceral digit short, *c*.5 long. Setae *dF* and *dG* subequal in length, *c*.20 long. Hypostome *c*.75 long. Each branch of peritremes 55–60 long. *Idiosoma*

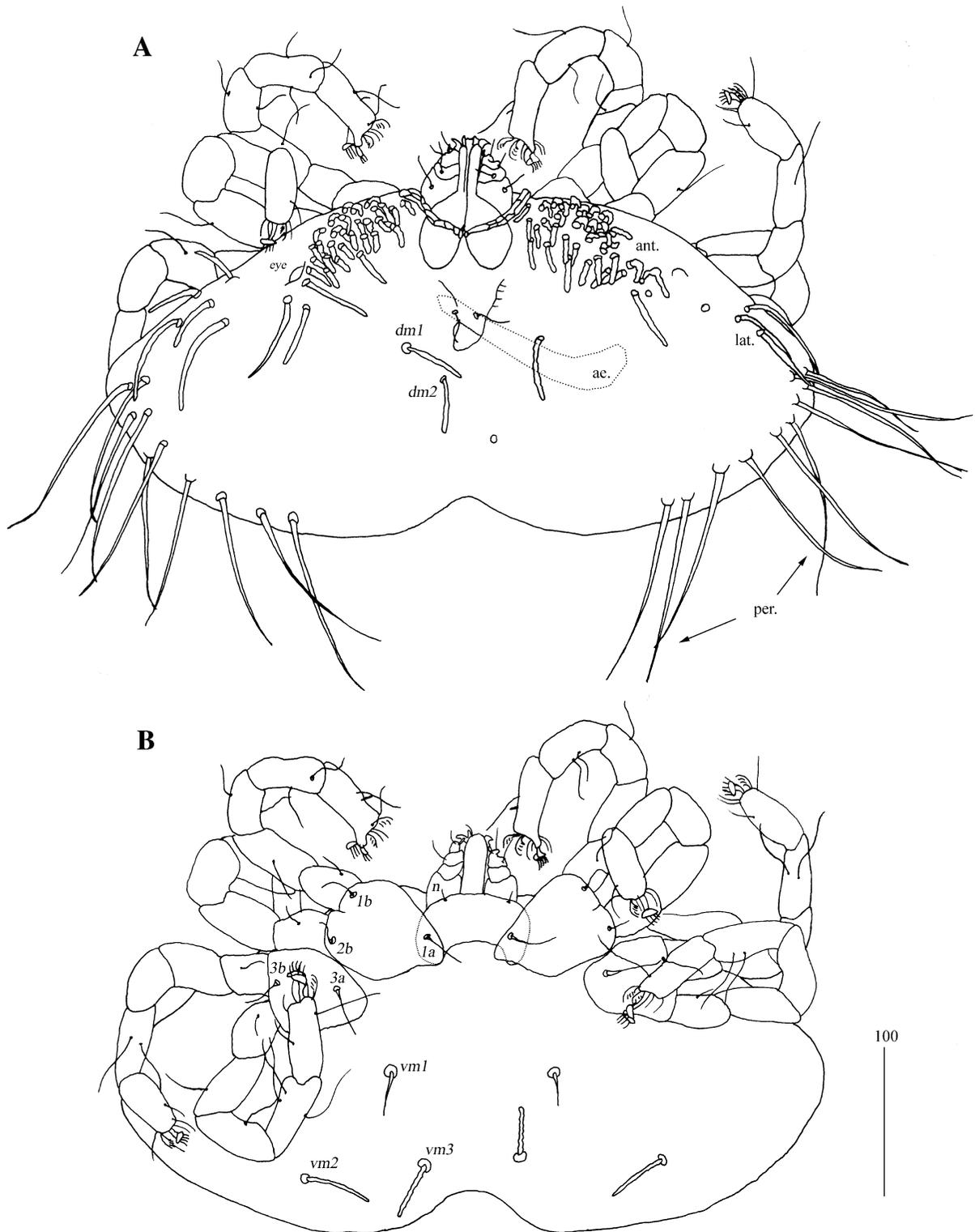


Fig. 4 *Pterygosoma pallidum* n. sp. Male. A, Dorsal view; B, Ventral view. Abbreviations: ae., aedeagus; ant., anterior setae; lat., lateral setae; per., peripheral setae

205–230 long, 450–455 wide. Dorsum with 3 groups of serrate setae: 31–33 pairs of anterior setae, 20–30 long; 7–9 pairs of lateral setae, 35–65 long, and 2 pairs of dorso-median setae: *dm1* and *dm2*, 30–40 long. Peripheral series represented by 9–10 pairs of smooth setae, 95–120 long. Eyes present. Aedeagus *c.*140 long, directed forward. Genito-anal opening with 1 pair of filiform setae situated anteriorly and 2 pairs of minute setae, *c.*5 long, situated posteriorly. Venter with 3 pairs of ventro-median setae: *vm1* filiform, *vm2–vm3* serrate. *Legs.* Coxal setation and setae of trochanters, femora and tibiae I–IV as in female. Setae *v'GII* and *v'GIII* present (in 2 paratypes setae *dFI* absent). All setae smooth except for serratesetae *dFI–IV* and slightly serrate setae *d'TiII–IV*, *v'TiII–IV* and *v''TiII–IV*. All tarsal setae as in female except for slightly serrate setae *vs'* of legs II.

Deutonymph [Based on 5 paratypes, Fig. 5.] *Gnathosoma*. Chelicerae 250–270 long, basal swollen part 105–110 long, slender distal part 150–165 long. Fixed cheliceral digit *c.*10 long. Setae *dF* and *dG* subequal in length, 50–55 long. Subcapitular setae *n* 25 long. Each branch of peritremes *c.*100 long. Hypostome 75–80 long. *Idiosoma* 385–485 long, 620–765 wide. Dorsum with serrate setae. Antero-lateral part of idiosoma with *c.*120 pairs of setae that increase in length from anterior to posterior part of idiosoma, 35–80 long. Medial part of idiosoma with 2 pairs of dorso-median setae: *dm1* and *dm2*, 55–65 long. Peripheral setae represented by 19–26 pairs of slightly serrate setae, 80–125 long, arranged in 2 rows: dorsal row of 2 pairs of setae (1 pair situated laterally and 1 pair situated near genital field) and terminal row of 17–24 pairs of setae. Eyes present. Venter with 3 pairs of ventro-median setae *vm1–vm3*. Setae *vm1* filiform, setae *vm2* and *vm3* serrate. Genital slit situated dorsally. Genital setal series represented by 3 pairs of setae *g1–g3*. Setae *g1* and *g2* minute, *c.*5 long, setae *g3* most conspicuous, *c.*30 long. Pseudanal setal series represented by 4–6 pairs of serrate setae *ps*. Coxal setation as in female; setae *1b* with barely discernible serration. *Legs* as in female, only additional setae *v''GI* present.

Protonymph [Based on 7 paratypes, Fig. 6.] *Gnathosoma* as in female. Chelicerae 80–100 long, swollen cheliceral part 40–55 long, slender distal part 35–45 long. Movable cheliceral digit without basal spur.

Fixed cheliceral digit *c.*10 long. Palpal femur with slightly serrate setae *dF*, 25–30 long; palpal genu with smooth setae *dG*, 25–30 long. Subcapitular setae *n* 10 long. Each branch of peritremes 60–75 long. Hypostome 60–80 long. *Idiosoma* 365–400 long, 630–670 wide. Dorsum with serrate setae. Antero-lateral part with 33–40 pairs of setae, 25–55 long. Medial part with 2 pairs of dorso-median setae *dm1* and *dm2*, 35–50 long. Peripheral setae represented by 21–28 pairs of slightly serrate setae, 85–125 long, arranged in 2 rows: dorsal row of 2 pairs (1 pair near lateral part of idiosoma, 1 pair near genital field) and terminal row with 19–26 pairs. Eyes present. Venter with 3 pairs of ventro-median setae: *vm1* filiform, *vm2* and *vm3* serrate. Genital slit situated mainly ventrally. Genital series represented by 3 pairs of minute genital setae *g1–g3*. Pseudanal setal series represented by 5–6 pairs of serrate setae *ps*. *Legs* as in female, only additional setae *v''GI* present.

Larva [Based on 8 paratypes, Fig. 7.] *Gnathosoma*. Chelicerae *c.*60 long, swollen cheliceral part 35 long, slender distal part 25 long. Fixed cheliceral digit *c.*5 long. Movable cheliceral digit without basal spur. Palpal femur and genu with setae *dF* and *dG*, 25–30 long. Subcapitular setae *n* absent. Each branch of peritremes *c.*30 long. Hypostome 45–50 long. *Idiosoma* 200–255 long, 280–335 wide. Dorsum with 11 pairs of slightly serrate setae, 15–30 long: 4 pairs situated anteriorly, 4 pairs medio-laterally and 3 pairs posteriorly. Eyes present. Venter without ventro-median setae *vm1–vm3*. Genital slit situated mainly ventrally. Genital setae absent. Pseudanal setal series represented by 2 pairs of slightly serrate setae *ps1* and *ps2*; setae *ps1* situated ventrally, setae *ps2* situated terminally. *Legs.* Coxal setation: *1a*, *1b*, *3a* arranged in formula 2–0–1, all setae filiform; setae of trochanters I–III (0–0–0), femora I–III (3–1–1), genua I–III (2–0–0), tibiae I–III (5–3–3). All setae on each podomere smooth, except for serrate setae *dFI–III*, *dTiII–III*, *v'TiII–III* and *v''TiII–III*. Setation of tarsi: I 11 setae (*ft*, *tc'*, *p'*, *it'*, *it''*, *a'*, *a''*, *u'*, *u''*, *vs'*, *vs''*) and solenidion *ω1*; II 8 setae (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*) and *ω1*; III and IV with 8 setae each (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*). Setae *it'* and *it''* of leg I in form of eupathidia; all setae *p'* and *p''* fan-like, setae *a'* and *a''* of legs I–III and setae *u'* and *u''* of leg I smooth; setae *ft*, *tc'*, *vs'*, *vs''* and *u'*, *u''* of legs I–III slightly serrate.

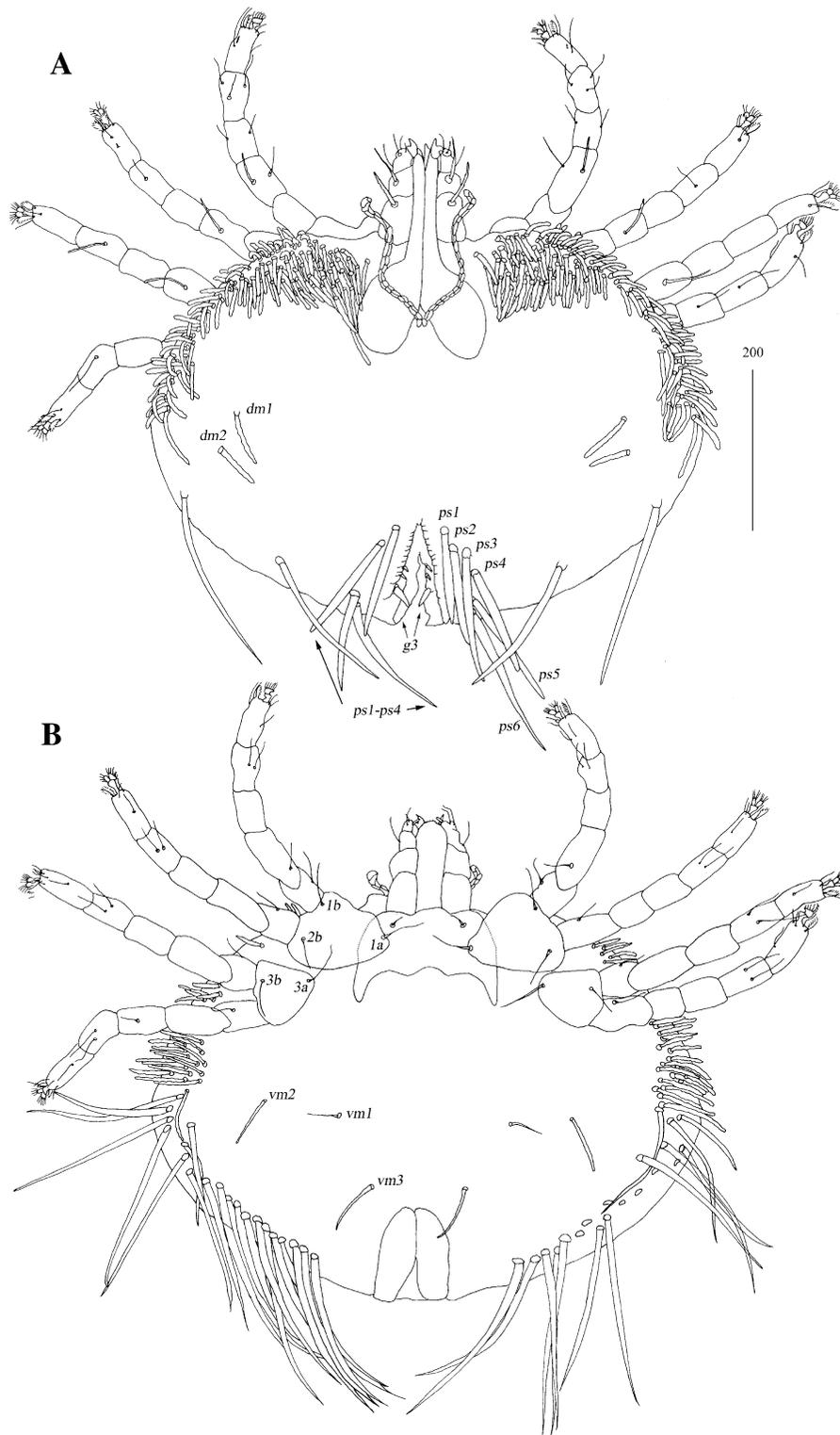


Fig. 5 *Pterygosoma pallidum* n. sp. Deutonymph. A, Dorsal view; B, Ventral view

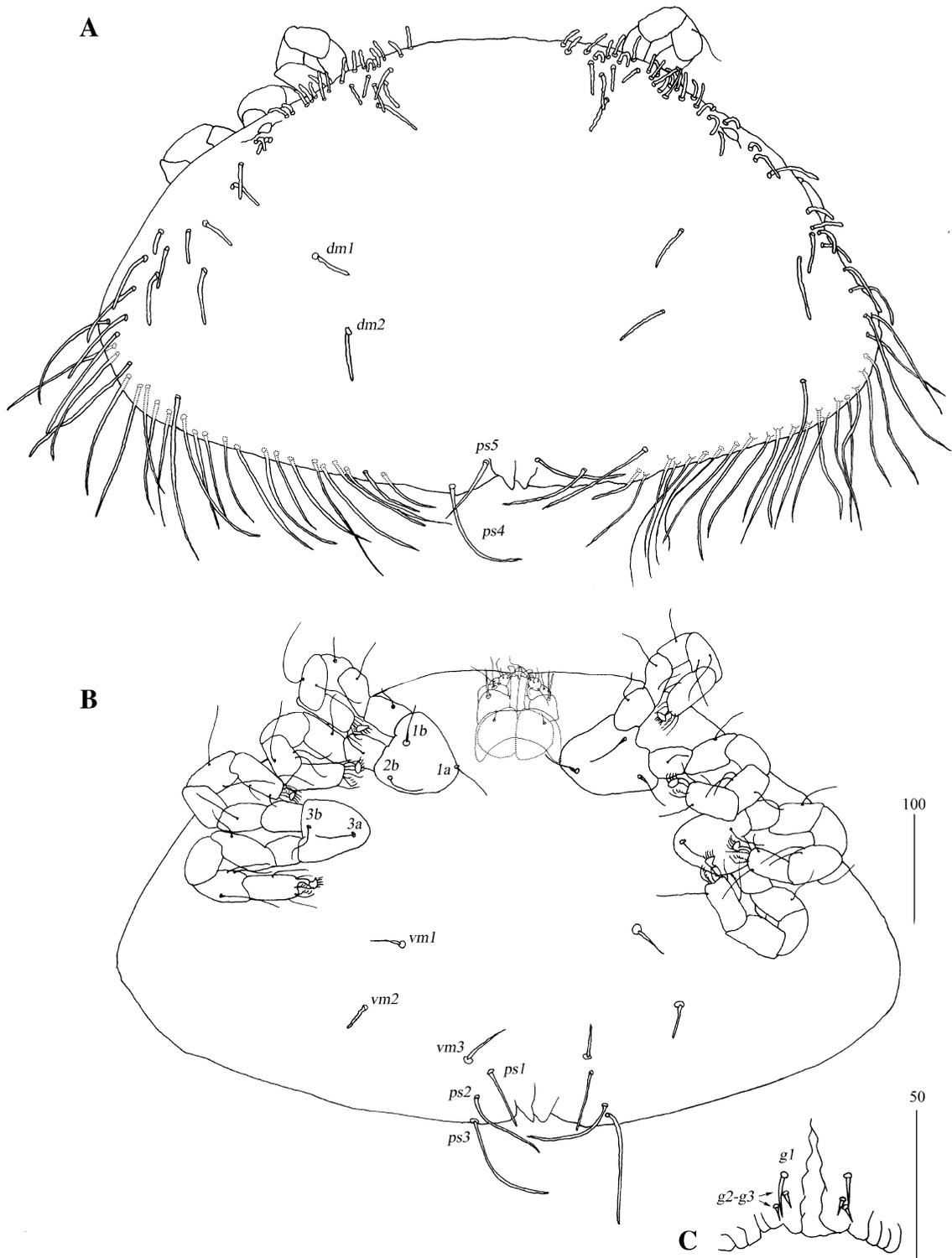


Fig. 6 *Pterygosoma pallidum* n. sp. Protonymph. A, Dorsal view; B, Ventral view; C, Genital region

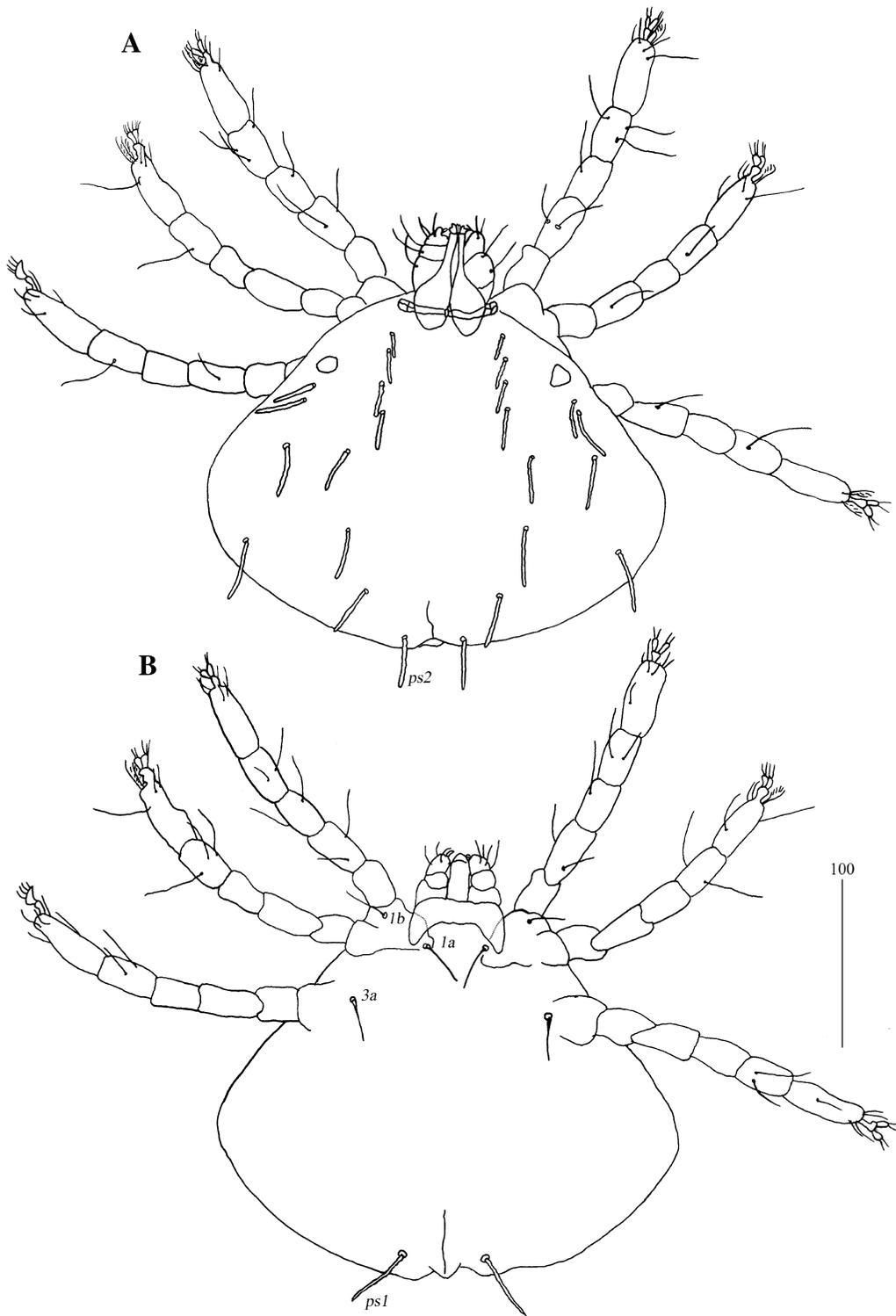


Fig. 7 *Pterygosoma pallidum* n. sp. Larva. A, Dorsal view; B, Ventral view

Differential diagnosis

This species is very similar to *Pterygosoma mutabilis* Jack, 1961 collected from *Trapelus mutabilis* (Merrem) from Libya (Jack, 1961). In females of both species, arrangement of the dorsal setae and the setation of the palps and tarsi, tibiae, femora and trochanters I–IV are the same, the swollen cheliceral part is shorter than the slender cheliceral part, the palp setae *dG* are longer than setae *dF*, and three pairs of genital setae are present. Females of the new species differ from *P. mutabilis* in the following features. In *P. pallidum* n. sp., eyes are present, the idiosomal dorso-lateral setae, dorso-median setae *dm1* and *dm2* and ventro-median setae *vm2* and *vm3* are serrate, palp seta *dF* is slightly serrate, the hypostomal apex has small teeth, setae *dGI* are absent or reduced to minute spine-like structures, leg setae *d'FI–IV* and *d'TiII–IV* are serrate, genital setae *g1–g3* are slightly serrate at the tip. In females of *P. mutabilis*, the eyes are absent, the dorsal-lateral setae are slightly serrate and setae *dm1* and *dm2* are smooth or with barely discernible serration, setae *vm2* and *vm3* are smooth, setae *dF* are smooth, the hypostomal apex is smooth, filiform setae *dGI* are present, leg setae *d'FI–IV* are slightly serrate, setae *d'TiII–IV* are smooth and genital setae *g1–g3* are spine-like.

Remarks

In 1962 Jack recognized six species groups for African mites of the genus *Pterygosoma* (*sensu stricto*): *transvaalense*, *hirsti*, *armatum*, *fimbriata*, *melanum* and *neumanni* (see Jack, 1962a). Diagnoses of these species groups are based on five characters of females, i.e. the length ratio of the swollen cheliceral part and slender distal part, the number of pseudanal setae *ps* (genital setae *sensu* Jack, 1962a), the location of setae *ps*, the number of genital setae (genital spines *sensu* Jack, 1962a) and the length to width ratio of setae *ps*. According to these characteristics, the new species does not belong to any of these groups. In *P. pallidum* n. sp., the swollen cheliceral part is shorter than the slender distal part (feature of the *melanum* and *neumanni* groups), a variable number (4–6 pairs) of pseudanal setae is present (character state within the range observed for all six groups), pseudanal setae are situated dorsally and ventrally (feature of the *transvaalense*, *hirsti*, *armatum* and *fimbriata* groups), three

pairs of genital setae are present (character state observed in the *transvaalense*, *melanum*, and *neumanni* groups) and pseudanal setae are much longer than broad (characteristic of *transvaalense* and *neumanni* groups).

Species group *inermis*

Pterygosoma parasiniatum n. sp.

Type-host: *Pseudotrapelus* cf. *sinaitus* (Heyden) (Sauria: Agamidae). This “population” represents two hybridizing species, *P. sinaitus* (*sensu stricto*) and an undescribed species, with mtDNA introgression from the first to the second (Melnikova et al., 2015).

Type-locality: Ma’an province, 20 km north-west of Al Mudawarra (ZISP 28780), Jordan (2012, coll. by D. Melnikov).

Type-material: Holotype female and paratypes (2 females, 6 males, 2 protonymphs and 4 larvae) are deposited in the ZISP (Reg. No. AVB 15-0910-002); and paratypes (3 females, 6 males, 3 protonymphs and 4 larvae) are deposited in the AMU (AMU-PTE20.1). All mites were collected by D. Melnikov.

Etymology: The name *parasiniatum* refers to the closely related species, *Pterygosoma sinaita*.

Representative DNA sequence: KT962107 (28S rDNA).

Description (Figs. 8–13)

Female [Based on the holotype and 5 paratypes, Figs. 8–10] *Gnathosoma*. Chelicerae 165 (145–165) long, swollen cheliceral part 75 (65–70) long, slender distal part 90 (80–90) long. Fixed cheliceral digit *c*.15 long, with minute tines. Movable cheliceral digit with basal spur. Palpal femur and genu with smooth setae *dF* and *dG* 30 (25–30) and 25 (20–25) long, respectively; palpal tibia with 3 smooth setae: *dTi*, *l'Ti*, *l''Ti* and short claw; palpal tarsi with 4 smooth setae. Subcapitulum with filiform setae *n*, 15 (10–15) long. Each branch of peritremes 80 (70–75) long. Hypostome 100 (100) long, with smooth rounded apex. *Idiosoma* 470 (400–500) long, 815 (530–850) wide. Anterio-lateral part of dorsum with 16 and 19 pairs of slightly serrate setae 20–30 long, on right and left side, arranged in 2 groups: anterior group consisting of 11 and 14 (12–15) setae, lateral group consisting of 4 (4–5) setae. One pair of slightly serrate setae, 35–45

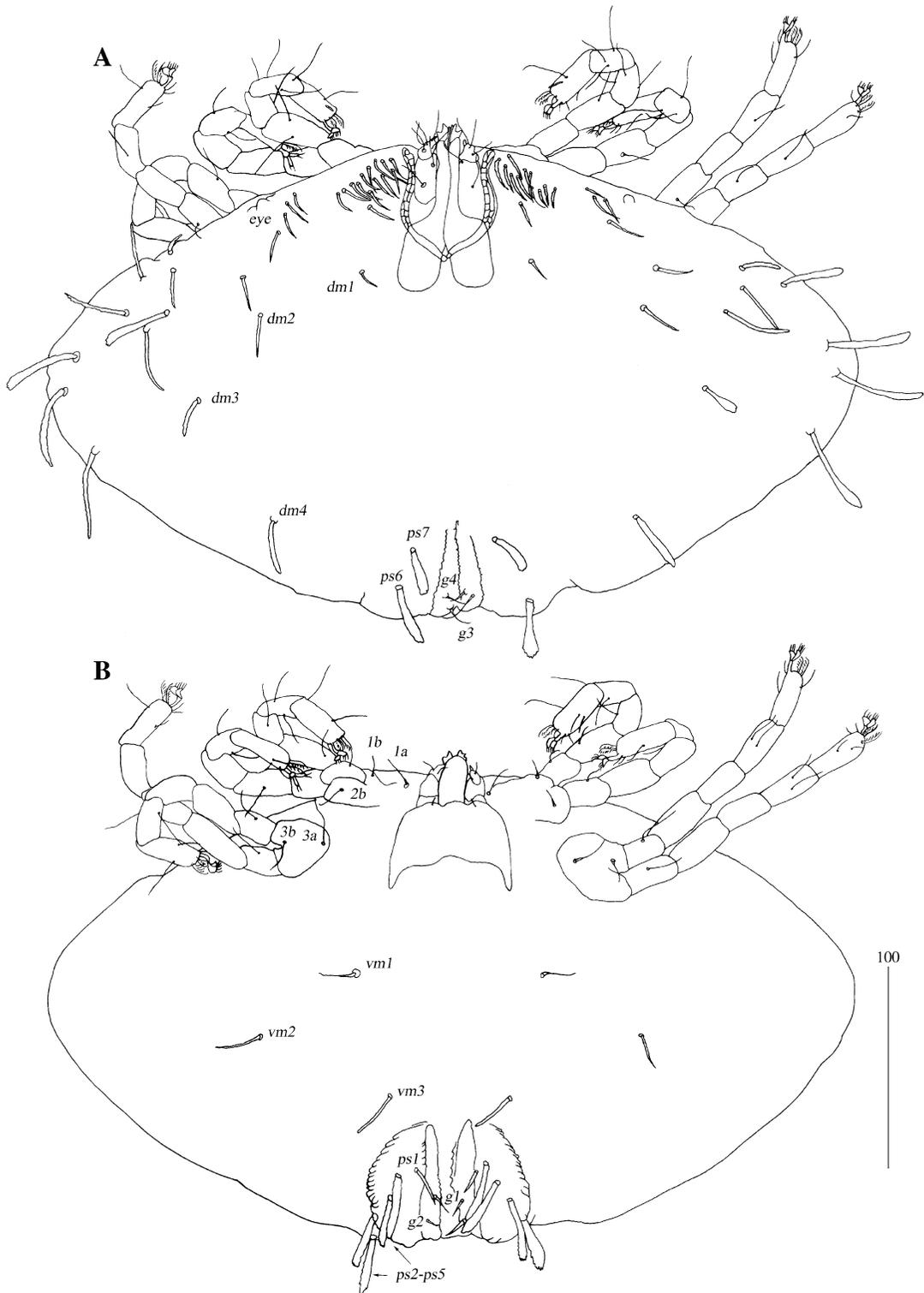


Fig. 8 *Pterygosoma parasiniatum* n. sp. Female. A, Dorsal view; B, Ventral view



Fig. 9 *Pterygosoma parasiniatum* n. sp. Female, shape of setae. A, Anterior seta; B, Dorsal femoral seta of leg I (*dFI*); C, Ventro-medial seta *vm2*; D, Smooth pseudanal seta *ps1*; E, Pseudanal slightly expanded seta *ps5*; F, Pseudanal paddle-shaped seta *ps6*; G, Postero-lateral seta

(30–35) long, present posterior to lateral groups. Medial part of idiosoma with 4 pairs of dorso-median setae *dm1–dm4*: 20 (20–25), 45 (40–45), 40 (45–50) and 55 (50–55) long, respectively. Setae *dm1* and *dm2* with barely discernible serration, setae *dm3* and *dm4* slightly expanded and with minute spicules on apical part. Postero-lateral part of idiosoma with 7 and 9 (6–10) pairs of setae of 2 types: 4 and 6 (4–7) pairs of slightly serrate or apically expanded shorter setae, 30–65 (30–70) long, on right and left side and 3 (2–3) pairs of paddle-shaped longer setae, 85–90 (80–85)

long, with minute spicules on apical part. Eyes present. Venter with 3 pairs of ventro-median setae *vm1–vm3*. Setae *vm1* filiform, *vm2* and *vm3* with barely discernible serration. Genital slit situated mainly ventrally. Genital setal series represented by 4 pairs of spine-like setae *g1–g4*. Pseudanal setal series represented by 1 pair of smooth or very slightly serrate setae *ps1*, 4 pairs of slightly apically expanded setae *ps2–ps5* and 2 pairs of paddle-shaped setae *ps6* and *ps7*. Setae *ps1–ps4* situated ventrally, setae *ps5* terminally, setae *ps6* and *ps7* dorsally. *Legs*. Coxal

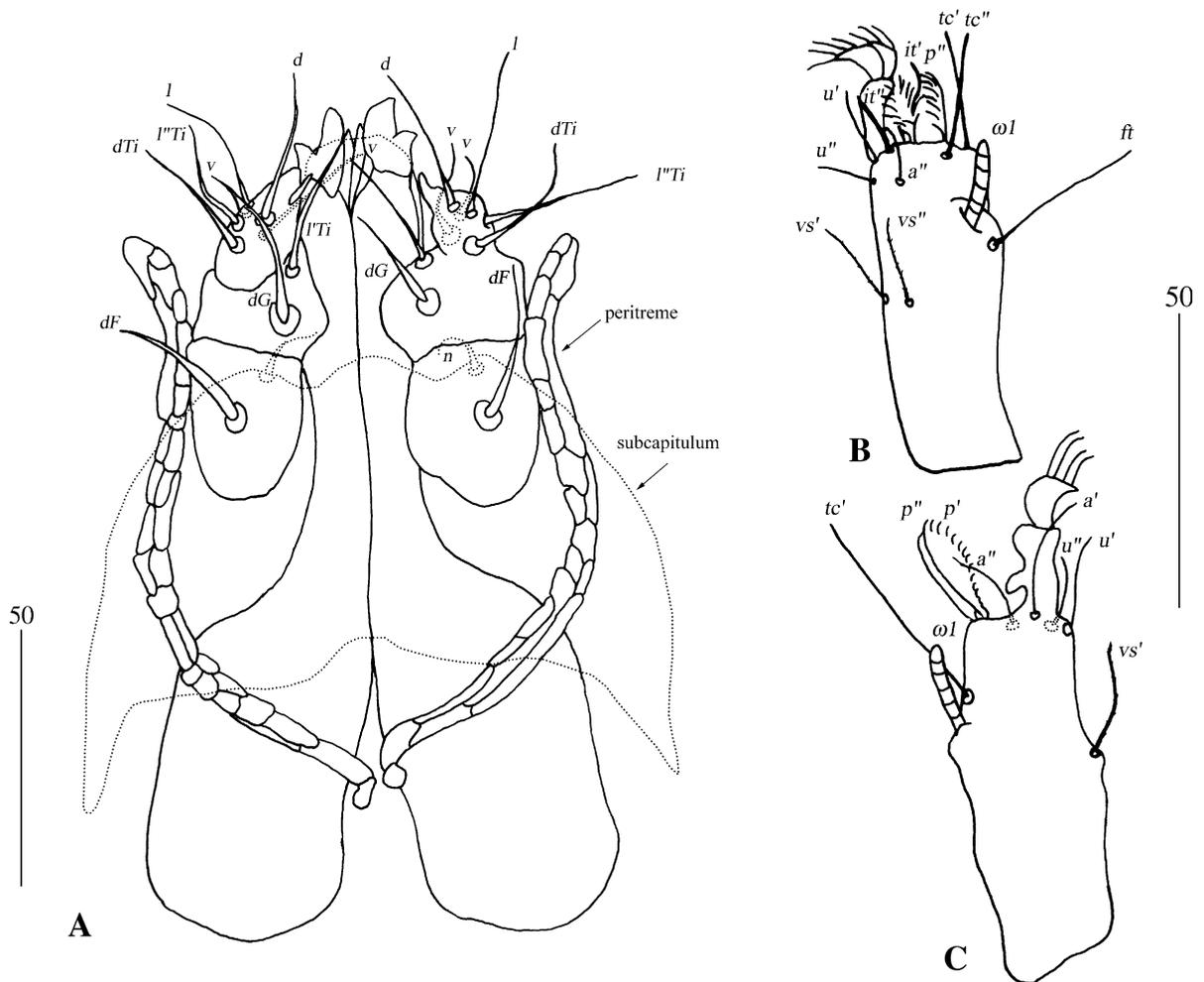


Fig. 10 *Pterygosoma parasiniatum* n. sp. Female, details. A, Gnathosoma, dorsal view; B, Tarsus I, ventro-lateral view; C, Tarsus II, lateral view. Setal notations: *v*, ventral; *d*, dorsal; *l*, lateral. Podomere abbreviations: *F*, femur; *G*, genu; *Ti*, tibia

setation: *1a*, *1b*, *2b*, *3a*, *3b* arranged in formula 2–1–2–0; all setae filiform. Setation of trochanters-tibiae corresponding to group 2 of Jack (1964): trochanters I–IV (1–1–1–1), femora I–IV (3–1–1–1), genera I–IV (3–0–0–1), tibiae I–IV (5–3–3–3). All setae on each podomere filiform, except for slightly plumose setae *d'Fl–III*, *dGl*, *v'TiI–IV* and *v''TiI–IV*. Setation of tarsi corresponding to group A of Jack (1964): I 13 setae (*ft*, *tc'*, *tc''*, *p'*, *p''*, *a'*, *a''*, *it'*, *it''*, *u'*, *u''*, *vs'*, *vs''*) and solenidion $\omega 1$; II 8 setae (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*) and $\omega 1$; III and IV with 8 setae each (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*). Setae *tc'*, *tc''*, *it'*, *it''* of leg I in form of eupathidia, *p'* and *p''* fan-like. Setae *tc'* of legs II–IV and setae *a'*, *a''*, *u'*, *u''* of legs I–IV smooth, setae *vs'* and *vs''* slightly serrate.

Male [Based on 12 paratypes, Fig. 11] *Gnathosoma* as in female. Chelicerae *c.*100 long, swollen cheliceral part and slender distal part subequal in length, *c.*50 long. Fixed cheliceral digit *c.*10 long. Hypostome 70–75 long. Each branch of peritremes *c.*65 long. *Idiosoma* 230–320 long, 420–515 wide. Dorsum with 3 groups of serrate setae: 14–18 pairs of anterolateral setae, 20–30 long; 3 pairs of dorso-median setae: *dm1–dm3* 35–40 long and 6–9 pairs of posterolateral setae, 25–60 long. Eyes present. Aedeagus 130–150 long, directed forward. Genito-anal opening with 2 pairs of filiform setae. Venter with 3 pairs of ventro-median setae: *vm1–vm2* filiform, *vm3* slightly serrate. *Legs* as in female except for absence of setae *v'TrIV*.

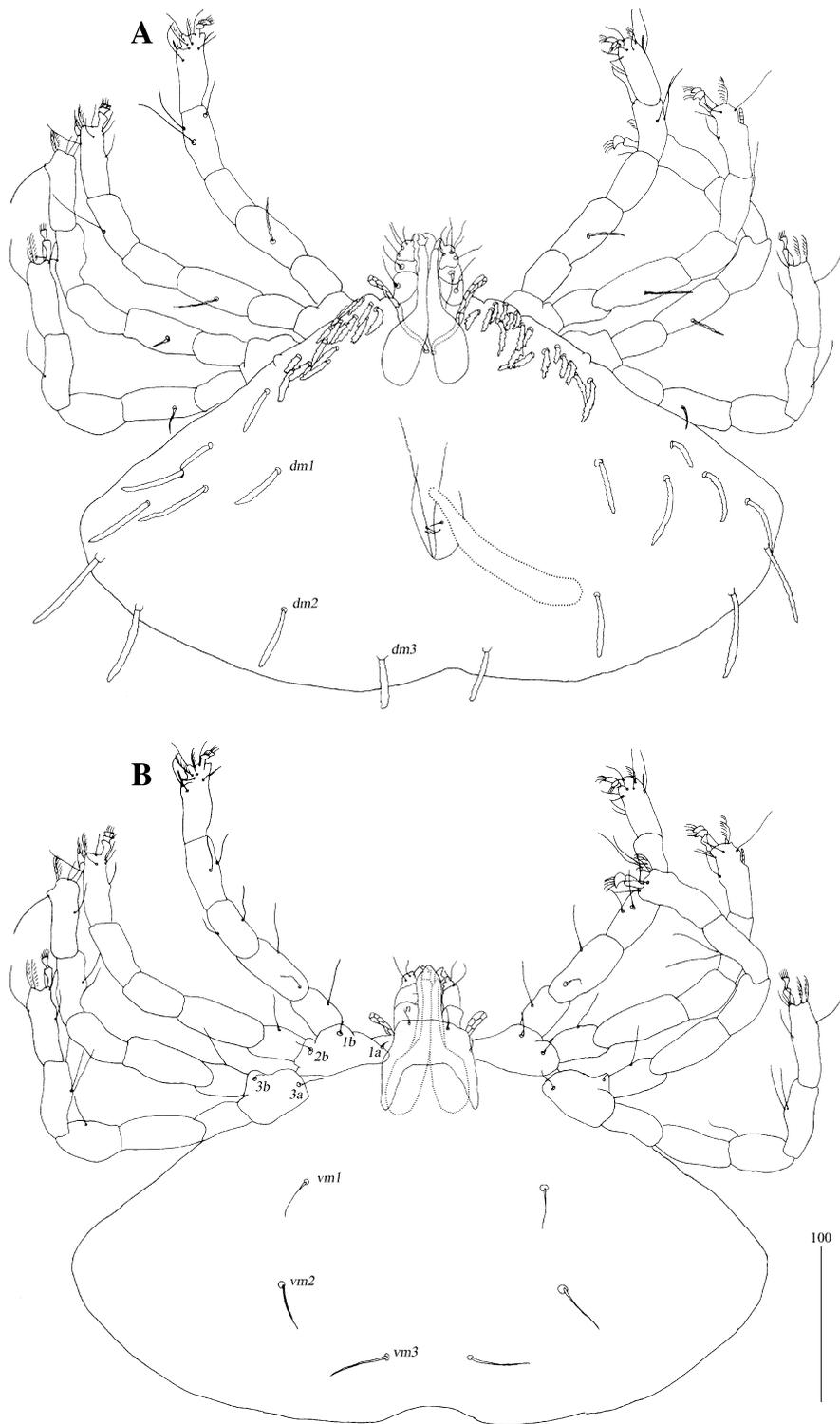


Fig. 11 *Pterygosoma parasiniatum* n. sp. Male. A, Dorsal view; B, Ventral view

Protonymph [Based on 5 paratypes, Fig. 12]. *Gnathosoma* as in female. Chelicerae 60–70 long, swollen cheliceral part and slender distal part subequal in length, 30–35 long. Fixed cheliceral digit 10–15 long. Setae *dF* and setae *dG* subequal in length, 15–20 long. Setae *dF* smooth, setae *dG* with barely discernible serration. Subcapitular setae *n* 10 long. Each branch of peritremes *c.* 65 long. Hypostome 80 long. *Idiosoma* 205–315 long, 380–530 wide. Dorsum with serrate setae. Anterior part of idiosoma with 9–10 pairs of setae, 20–30 long, arranged in 2 groups: anterior group consisting of 6–7 setae, lateral group consisting of 3 setae. One pair of serrate setae, 25–30 long, present posterior to lateral groups. Medial part of idiosoma with 4 pairs of dorso-median setae *dm1–dm4*. Setae *dm1* and *dm2* 20–25 long, setae *dm3* and *dm4* 30–35 long. Postero-lateral part of idiosoma with 8–9 pairs of setae, 25–50 long. Eyes present. Venter with 3 pairs of ventro-median setae: *vm1* filiform, *vm2* and *vm3* slightly serrate. Genital slit situated ventrally. Genital setal series represented by 3 pairs of genital setae *g1–g3*: setae *g1* 10 long, setae *g2* and *g3* *c.* 5 long. Pseudanal setal series represented by 5 pairs of setae *ps1–ps5*. Setae *ps1–ps3* smooth or slightly serrate, 10–20 long; setae *ps4* and *ps5* serrate, 25–30 long. *Legs* as in female, except for absence of setae *v'TrI*. Setae *dFI* smooth.

Larva [Based on 8 paratypes, Fig. 13] *Gnathosoma* as in female. Chelicerae 60–70 long, swollen cheliceral part and slender distal part equal in length 30 or 35 long. Fixed cheliceral digit *c.* 5 long. Setae *dF* and *dG* *c.* 25 and 20 long, respectively. Subcapitular setae *n* absent. Each branch of peritremes *c.* 30 long. Hypostome 60 long. *Idiosoma* 175–230 long, 210–300 wide. Dorsum with 11 pairs of slightly serrate setae, 20–30 long: 4 pairs situated anteriorly, 4 pairs medially and 3 pairs posteriorly. Eyes present. Venter without ventro-median setae *vm1–vm3*. Genital slit situated ventrally. Genital setae absent. Pseudanal setal series represented by 2 pairs of slightly serrate setae *ps1* and *ps2*; setae *ps1* situated ventrally, setae *ps2* situated terminally. *Legs*. Coxal setation: *1a*, *1b*, *3a* arranged in formula 2–0–1, all setae filiform; setae of trochanters I–III (0–0–0), femora I–III (3–1–1), genua I–III (2–0–0), tibiae I–III (5–3–3). All setae on each podomere slightly plumose, except for smooth setae *vFI*, *l'GI*, *l'TiI* and *l''TiI*. Setation of tarsi: I 11 setae (*ft*, *tc'*, *p'*, *it'*, *it''*, *a'*,

a'', *u'*, *u''*, *vs'*, *vs''*) and solenidion ωI ; II 8 setae (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*) and ωI ; III and IV with 8 setae each (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*). Setae *it'* and *it''* of leg I represented by eupathidia; setae *p'* and *p''* fan-like, setae *a'* and *a''* of legs I–III and setae *u'* and *u''* of leg I smooth; setae *ft*, *tc'*, *vs'*, *vs''* and *u'*, *u''* of legs I–III slightly serrate.

Note: It is worth mentioning that in all larval paratypes additional unpaired seta *ps* observed on the right side.

Differential diagnosis

This new species is most closely related to *Pterygosoma sinaita* Jack, 1961 collected from *Pseudotrappelus sinaitus* (Heyden) from south-west Arabia (Jack, 1961). Females of both species share the following character states: presence of eyes, arrangement of the dorsal setae and setation of legs I–IV, except for genua IV, tines on fixed cheliceral digit, longer setae *dF* in relation to *dG*, four pairs of smooth setae on palp tarsi, apically expanded setae *dm3*, four pairs of genital setae, smooth or slightly serrate setae *ps1*. Females of the new species differ from *P. sinaita* in the following features. In *P. parasiniatum* n. sp., the lateral group of the dorsal setae is represented by 4–5 pairs of setae and only one slightly serrate seta is present posterior to this group; 7–10 pairs of postero-lateral setae are present; the swollen cheliceral part is shorter than the slender distal part; subcapitular seta *n* is present; the hypostomal apex is smooth and rounded; setae *dm1* and *dm2* are with barely discernible serration; setae *v'GIV* are present and seven pairs of pseudanal setae *ps1–ps7* are present. In females of *P. sinaita*, the lateral group of the dorsal setae is represented by 10 pairs of setae and two filiform setae are present posterior to this group; six pairs of postero-lateral setae are present; the swollen cheliceral part and the slender distal part are equal in length; subcapitular seta *n* is absent; the hypostomal apex is flattened; setae *dm1* are filiform, setae *dm2* apically expanded, setae *v'GIV* are absent and six pairs of pseudanal setae *ps1–ps6* are present.

Pterygosoma theobaldi n. sp.

Type-host: *Phrynocephalus theobaldi* Blyth (Sauria: Agamidae).

Type-locality: Lake Pangong, Himalaya, India (ZISP 28778; 21.x.2013; coll. by the members of the Second

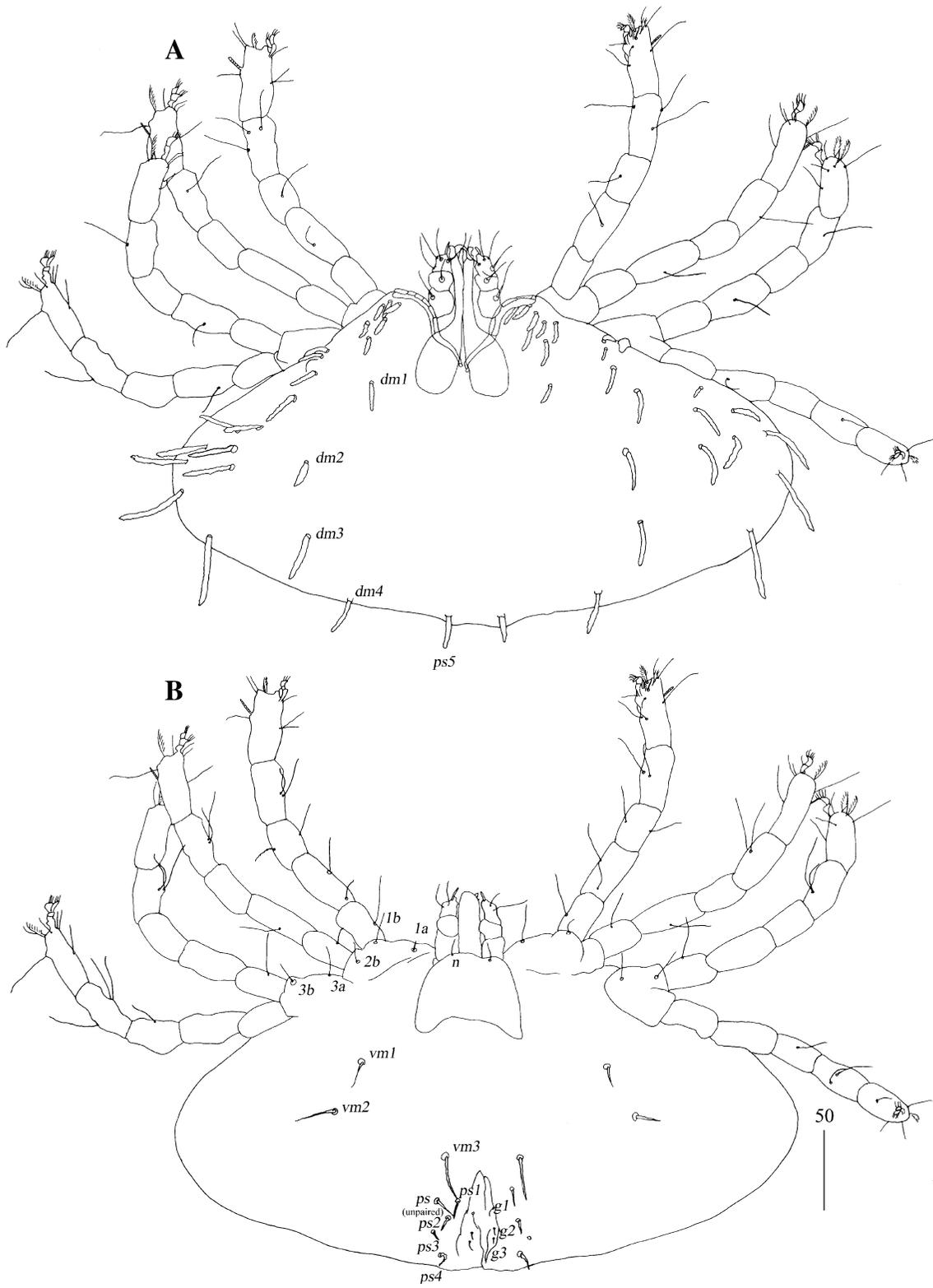


Fig. 12 *Pterygosoma parasiniatum* n. sp. Protonymph. A, Dorsal view; B, Ventral view

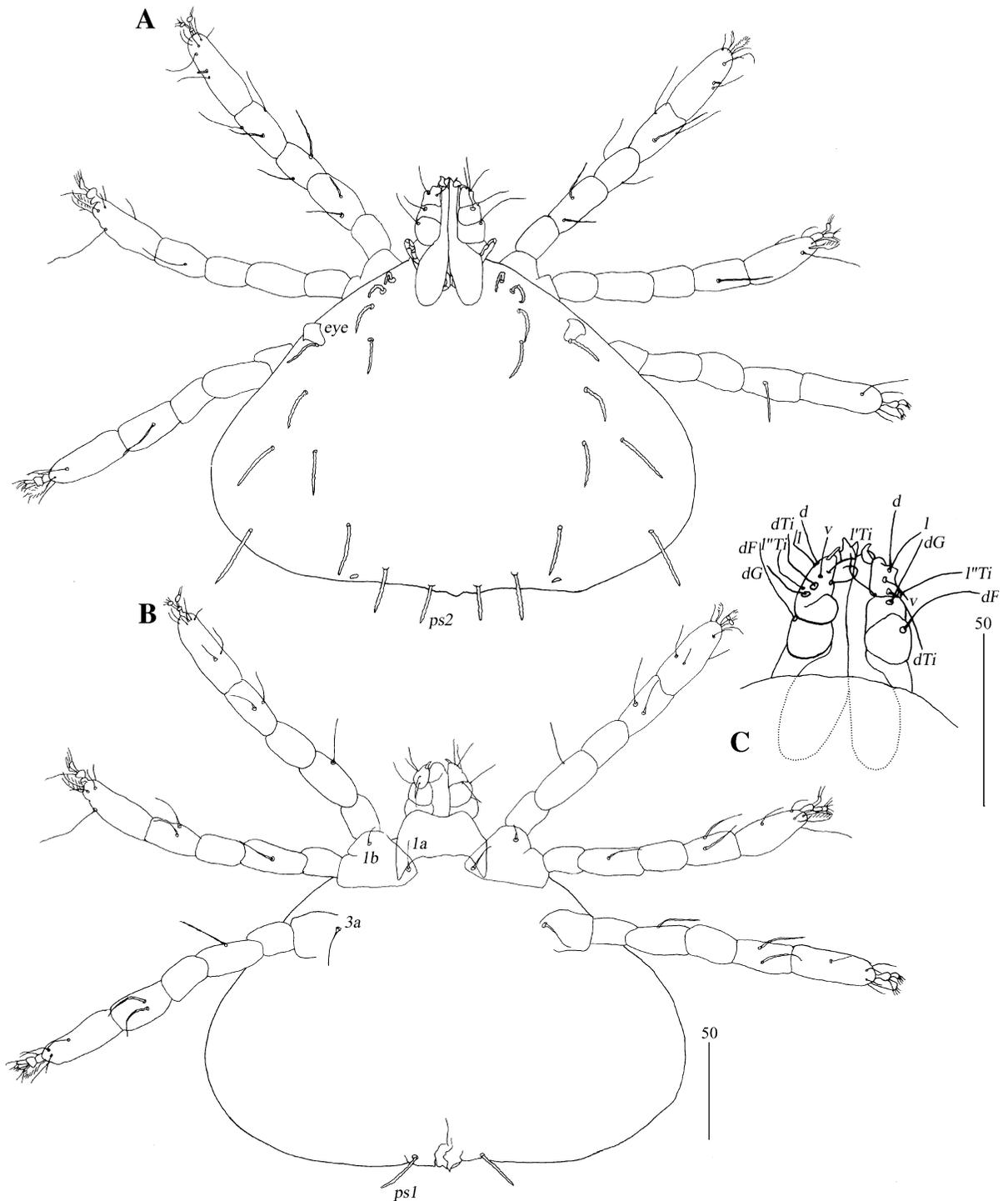


Fig. 13 *Pterygosoma parasiniatum* n. sp. Larva. A, Dorsal view; B, Ventral view; C, Gnathosoma, dorsal view. Setal notations: v, ventral; d, dorsal; l, lateral. Podomere abbreviations: F, femur; G, genu; Ti, tibia

West Himalayan Expedition arranged by the St. Petersburg Association of Scientists & Scholars).

Type-material: Holotype female (Reg. No. AMU-PTE21.1) and 4 female paratypes are deposited in the ZISP (Reg. No. ZISP AVB 15-0910-003); 9 female and 1 male paratypes are deposited in AMU (Reg. No. AMU-PTE21.1). All mites were collected by D. Melnikov.

Etymology: The specific epithet *theobaldi* is after the species name of the host.

Representative DNA sequences: KT962103 (*cox1*); KT962106 (28S rDNA).

Description (Figs. 14–16)

Female [Based on the holotype and 13 paratypes, Figs 14–15] *Gnathosoma*. Chelicerae 110 (100–115) long, basal swollen part and slender distal part subequal in length, *c.*55 (50–60) long. Fixed cheliceral digit *c.*15 long, with several tines. Movable cheliceral digit with basal spur. Palpal femur and genu with smooth dorsal setae *dF* and *dG* each: 25 (20–25) and 20 (20–25) long, respectively. Palpal tibia with 3 smooth setae: *dTi*, *l'Ti*, *l''Ti* and short claw. Palpal tarsi with 3 smooth setae. Subcapitular setae *n* absent. Each branch of peritremes *c.*55 (55–60) long. Hypostome 50 (50–60) long, with slightly flattened apex. *Idiosoma* 405 long (335–465), 720 wide (635–795). Anterior part of dorsum with 8–9 pairs of serrate setae, 20–25 (20–25) long, arranged in 2 groups: anterior group consisting of 3 (3) setae and lateral group consisting of 6 (4–7) setae. One pair of slightly serrate setae, 20 (20–25) long, present posterior to lateral groups. Medial part of idiosoma with 4 pairs of dorso-medial setae *dm1–dm4*, 25–30 (20–35) long. Setae *dm1* and *dm2* slightly serrate, setae *dm3* and *dm4* serrate. Postero-lateral part of idiosoma with 5 and 6 (4–6) serrate setae, 35–55 long, situated on right and left side of idiosoma, respectively. Eyes present. Venter with 3 pairs of ventro-medial setae *vm1–vm3*. Setae *vm1* filiform, *vm2* and *vm3* serrate. Genital slit situated terminally. Genital setal series represented by 4 pairs of spine-like setae *g1–g4*, 20–25 long. Pseudanal setal series represented by 1 pair of smooth or very slightly serrate setae *ps1*, *c.*20 long and 4 pairs of paddle-shaped setae *ps2–ps5*, 30–40 long. Setae *ps1* and *ps2* situated ventrally, *ps3* terminally, *ps4* and *ps5* dorsally. **Legs.** Coxal setation: *1a*, *1b*, *2b*, *3a*, *3b* arranged in formula 2–1–2–0; all setae filiform. Setae

of trochanters I–IV (1–1–1–1), femora I–IV (3–1–1–1), genua I–IV (2–0–0–0), tibiae I–IV (5–3–3–3). All setae on each podomere filiform, except for slightly serrate setae *v'TiII–IV* and *v''TiII–IV*. Setation of tarsi corresponding to group A of Jack (1964): I 13 setae (*ft*, *tc'*, *tc''*, *p'*, *p''*, *a'*, *a''*, *it'*, *it''*, *u'*, *u''*, *vs'*, *vs''*) and solenidion *ω1*; II 8 setae (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*) and *ω1*; III and IV with 8 setae each (*tc'*, *p'*, *p''*, *a'*, *a''*, *u'*, *u''*, *vs'*). Setae *tc'*, *tc''*, *it'*, *it''* of leg I in form of eupathidia, *p'* and *p''* fan-like. Setae *tc'* of legs II–IV and setae *a'*, *a''*, *u'*, *u''* of legs I–IV smooth, setae *vs'* and *vs''* of legs II–IV slightly serrate.

Male [Based on 1 paratype, Fig. 16] *Gnathosoma* as in female. Chelicerae 70 long, swollen cheliceral part and slender distal part subequal in length, *c.*35 long. Fixed cheliceral digit short, *c.*5 long. Hypostome 50 long. Each peritremal branch *c.*45 long. *Idiosoma* 180 long, 275 wide. Dorsum with 3 groups of serrate setae: 6 and 7 antero-lateral setae (10–15 long) on right and left side of idiosoma, respectively; 3 pairs of dorso-medial setae: *dm1–dm3* (20–25 long) and 5 pairs of postero-lateral setae (20–40 long). Eyes present. Aedeagus *c.*110 long, directed forward. Genito-anal opening with 2 pairs of filiform setae. Venter with 3 pairs of ventro-medial setae: *vm1* filiform, *vm2* and *vm3* serrate. **Legs** as in female, except for absence of setae *v'TrIV*. All setae on each podomere filiform, except for slightly serrate setae *dFI–II* and *v'TiIII–IV*, *v''TiIII–IV*. Setae *vs'* of tarsi III–IV slightly serrate.

Differential diagnosis

This new species is most closely related to *Pterygosoma inermis* Trägårdh, 1905 collected from *Laudakia stellio* (Linnaeus) in Egypt (Trägårdh, 1905). In females of both species, the idiosomal size (length 335–465, width 635–795) and the arrangement of the dorsal setae are very similar, the number of setae on genua I–III and tibiae, femora and tarsi I–IV is the same, four pairs of setae are present on the palp tarsi, setae *dF* are longer than setae *dG*, four pairs of genital setae and five pairs of pseudanal setae are present, setae *ps1* are smooth and setae *vm1* are filiform. Females of this new species differ from *P. inermis* by the following features. In females of *P. theobaldi* n. sp., eyes are present, subcapitular setae *n* are absent, the swollen cheliceral part and the slender distal part are subequal in length, the fixed cheliceral digit has

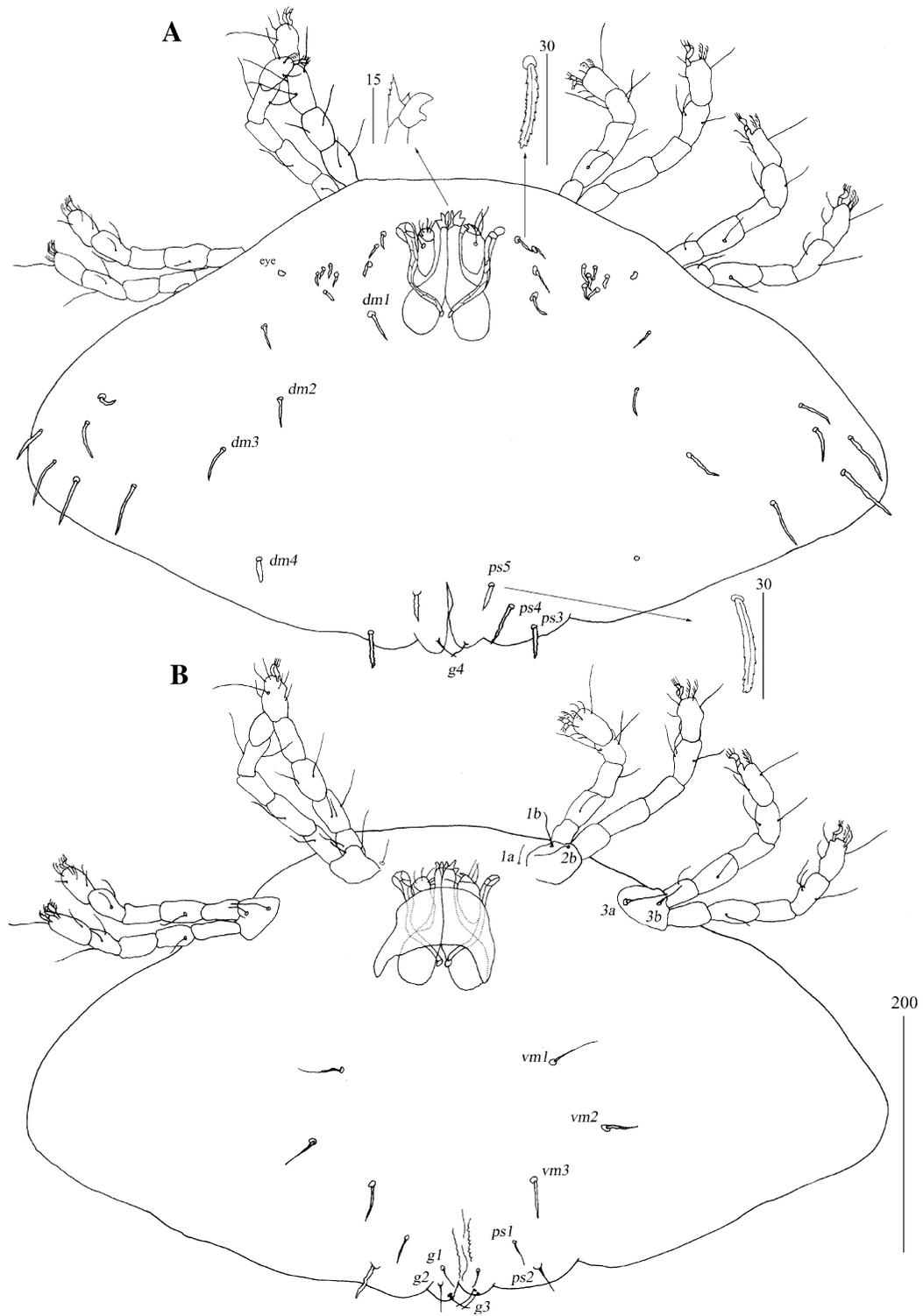


Fig. 14 *Pterygosoma theobaldi* n. sp. Female. A, Dorsal view; B, Ventral view

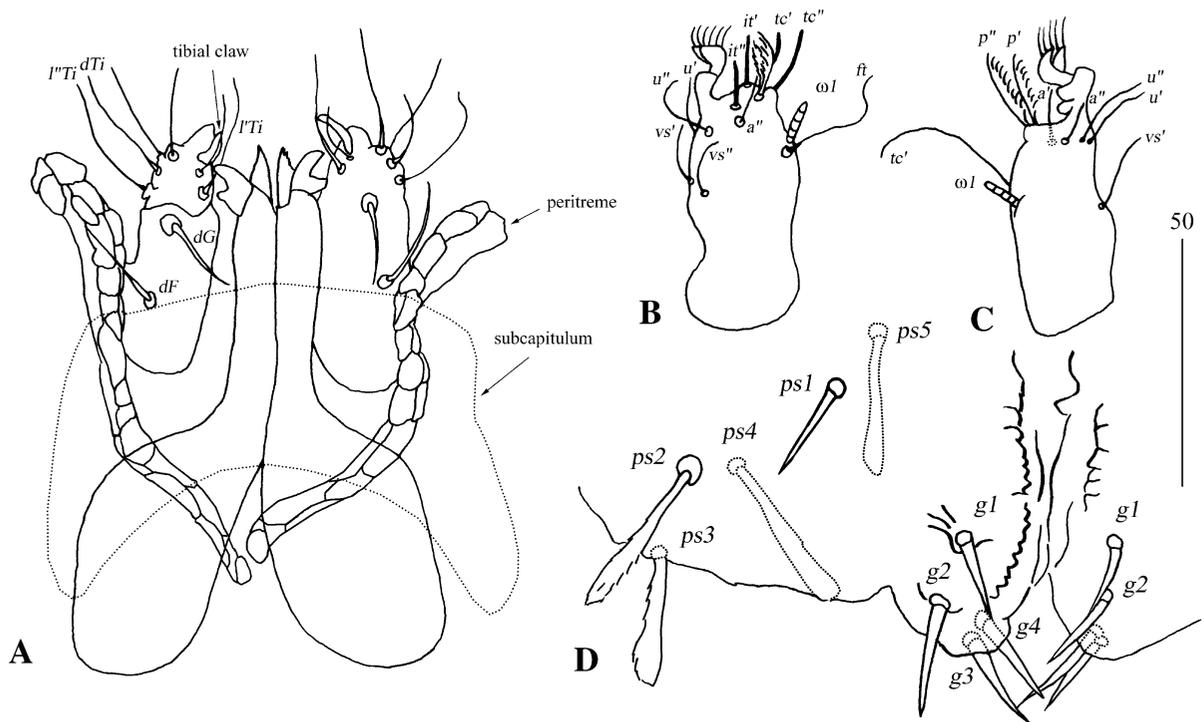


Fig. 15 *Pterygosoma theobaldi* n. sp. Female, details. A, Gnathosoma, dorsal view; B, Tarsi I, lateral view; C, Tarsi II, lateral view; D, Genital region. Setal notations: *d*, dorsal; *l*, lateral Podomere abbreviations: *F*, femur; *G*, genu; *Ti*, tibia

small tines, dorso-median setae *dm1* and *dm2* are slightly serrate, ventro-median setae *vm2–vm3* and pseudanal setae *ps1–ps5* are serrate and leg setae *vGIV* are absent. In females of *P. inermis*, eyes are absent, setae *n* are present, the swollen cheliceral part is longer than the slender part, the fixed cheliceral digit is spinous, dorso-median setae *dm1* and *dm2* and ventro-median setae *vm2* and *vm3* are filiform, setae *ps1–ps5* are apically expanded and leg setae *vGIV* are present.

Barcode analyses

We analyzed 601 bp fragment of the *cox1* gene fragment for five specimens of *P. theobaldi* n. sp. (all of the same haplotype) and 626 bp fragment of the same region for three specimens of *P. pallidum* n. sp. (also all of one haplotype). The analysis of the *cox1* of *P. parasiniatum* yielded ambiguous results thus they are not included here (probably we amplified a pseudogene sequence fragment as translation to the

amino acid sequence resulted in multiple stop codons in all genetic frames and codes applied).

Comparison of the sequences from *P. pallidum* and *P. theobaldi* showed 17 amino acid substitutions. The mean distance between *cox1* sequences of these two species was 20.1% (standard deviation 0.11%).

Nuclear sequence data, including *c.*800 nucleotide positions for the D2 region of the 28S rDNA, were obtained for four specimens of *P. pallidum*, three specimens of *P. theobaldi* and one specimen of *P. parasiniatum*. There was no intraspecific variation in this sequence region, but sequences were substantially different between the species studied.

Results of the present study shows that the morphological dissimilarities between the described species are well supported by the genetic distances between nucleotide sequences, i.e. genetic distances between D1–D2 region sequences of *P. pallidum* n. sp. and species from the *inermis* group (*P. parasiniatum* n. sp. and *P. theobaldi* n. sp.) were 15.4 and 19.4%, respectively, whereas the distance between the species of the *inermis* group was 8.1%.

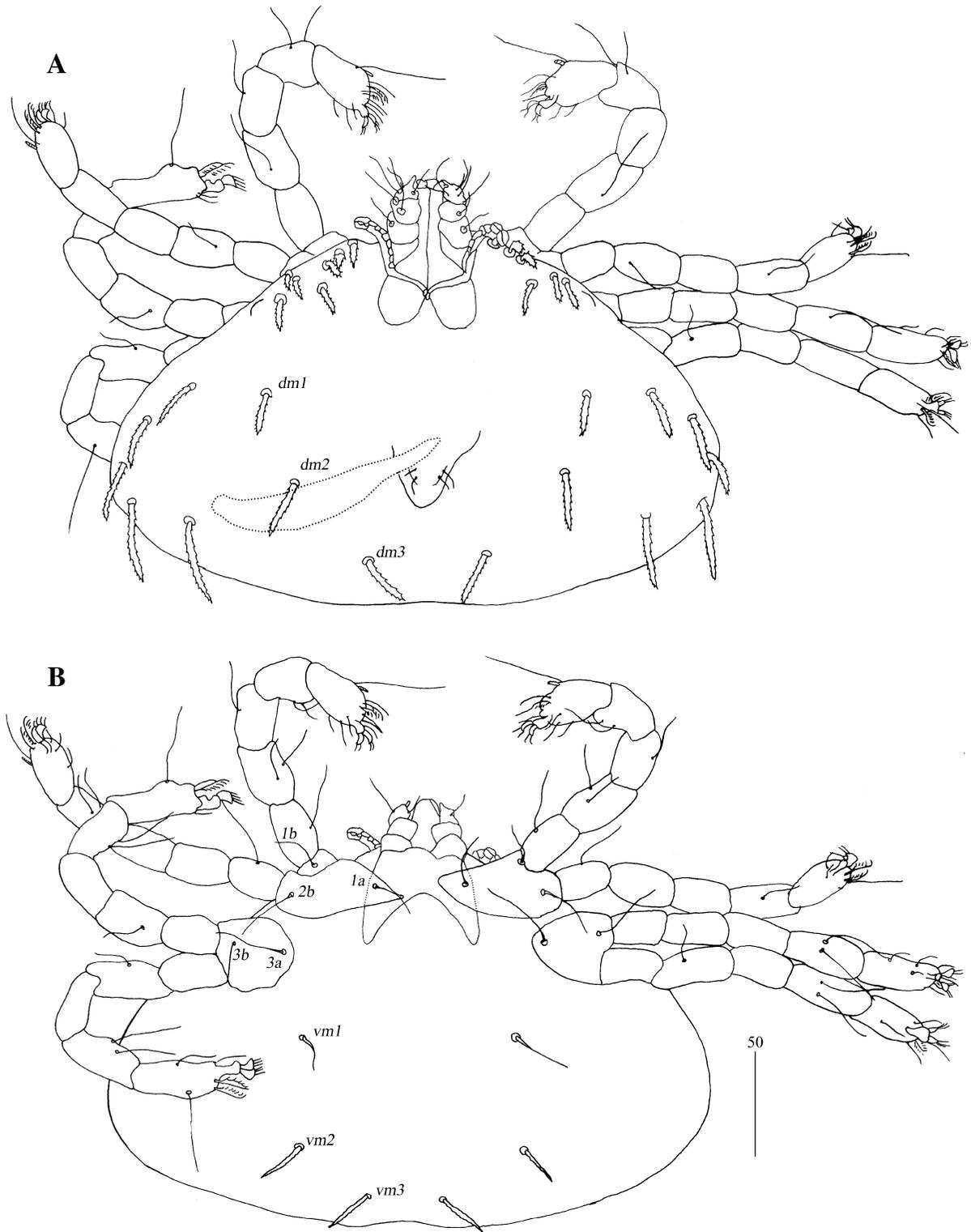


Fig. 16 *Pterygosoma theobaldi* n. sp. Male. A, Dorsal view; B, Ventral view

Key to the species of *inermis* species group based on female morphology

The description of *P. inermis stoliczkan*a Jack, 1962 is vague; the type-specimens were not available for our study and this subspecies has never been recollected from the type-host since the original description. Therefore we do not include this subspecies in the key.

- 1a Leg setae *vGII* and *vGIII* absent. Setae *dm3* present 2
- 1b Leg setae *vGII* and *vGIII* present. Setae *dm3* absent *P. tuberculata* Jack, 1962
- 2a Palp tarsi with 3 setae. Swollen cheliceral part equal, subequal to or longer than slender distal part 4
- 2b Palp tarsi with 4 setae. Swollen cheliceral part shorter than slender distal part 3
- 3a Antero-dorsal setal series with 4–5 pairs. Eyes absent. Setae *dF* and *dG* subequal in length. Setae *vm2* and *vm3* smooth. Pseudanal series with 5 pairs of setae *ps* *P. adramitana* Jack, 1961
- 3b Antero-dorsal setal series with 12–15 pairs. Eyes present. Setae *dF* longer than *dG*. Setae *vm2* and *vm3* slightly serrate. Pseudanal series with 7 pairs of setae *ps* *P. parasiniatum* n. sp.
- 4a One pair of smooth setae present posterior to lateral group of dorsal setae. Pseudanal series with 5 pairs of setae *ps* 5
- 4b Two pairs of smooth or slightly serrate setae present posterior to lateral group of dorsal setae. Pseudanal series with 6–7 pairs of setae *ps* 6
- 5a Fixed cheliceral digit spinous. Setae *ps2–ps5* apically expanded. Setae *vm2* and *vm3* smooth. Subcapitular setae *n* present..... *P. inermis* Trägårdh, 1905
- 5b Fixed cheliceral digit with tines. Setae *ps2–ps5* serrate. Setae *vm2* and *vm3* serrate. Subcapitular setae *n* absent *P. theobaldi* n. sp.
- 6a Subcapitular setae *n* absent. Postero-lateral row with 2 or 6 pairs of setae. Pseudanal series with 6 pairs of setae *ps*. Setae *vm2* and *vm3* smooth. Setae *vGIV* absent 7
- 6b Subcapitular setae *n* present. Postero-lateral row with 9–10 pairs of setae. Pseudanal series with 7 pairs of setae *ps*. Setae *vm2* and *vm3* slightly

- serrate. Setae *vGIV* present *P. aqabensis* Fajfer & Melnikov, 2014
- 7a Fixed cheliceral digit spinous. Setae *dF* shorter than *dG*. Setae *dm1* *P. dhofarensis* Fajfer & Melnikov, 2014
- 7b Fixed cheliceral digit with tines. Setae *dF* longer than *dG*. Setae *dm1* smooth..... *P. sinaita* Jack, 1961

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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