Combined description (morphology with DNA barcode data) of a new quill mite *Torotrogla paenae* n. sp. (Acariformes: Syringophilidae) parasitising the Kalahari scrub-robin *Cercotrichas paena* (Smith) (Passeriformes: Muscicapidae) in Namibia

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**Abstract** A new quill mite species *Torotrogla paenae* n. sp. (Acariformes: Syringophilidae) parasitising the Kalahari scrub-robin *Cercotrichas paena* (Smith) (Passeriformes: Muscicapidae) in Namibia is described based on the external morphology and DNA barcode data (the mitochondrial cytochrome *c* oxidase subunit 1 sequences, *cox1*). Females of *T. paenae* n. sp. morphologically differ from the most similar species *T. lusciniae* Skoracki, 2004 by the total body length (780–830 vs 645–715 μm in *T. lusciniae*) and the presence of hysteronotal shields (vs absence), apunctate propodonotal and pygidial shields (vs punctate), apunctate coxal fields (vs punctate), the fan-like setae *p*’ and *p”* of legs III–IV provided with c.10 tines (vs 14–15) and the length of setae *si* (140–180 vs 190–210 μm) and *se* (160–185 vs 210–225 μm). The male of *T. paenae* n. sp. morphologically differs from *T. lusciniae* by the lateral branch of peritremes composed of 4 chambers (vs 7–8 chambers) and lengths of setae *ve* (45 vs 70–75 μm) and *se* (120 vs 165 μm).

**Introduction**

Quill mites (Acariformes: Syringophilidae) are tiny, permanent bird ectoparasites inhabiting quills of feathers. Syringophilids spend there all their life-cycle and also feed by piercing quill walls with dagger-like chelicerae and suck liquid components of surrounding soft tissue (Casto, 1976; Skoracki, 2011). The family is presently represented by 338 species belonging to 60 genera, which were recorded from 478 bird species from 93 families and 24 orders (Glowska et al., 2015a; Zmudzinski & Skoracki, 2017). The genus *Torotrogla* Kethley, 1970 is widespread globally with proven occurrence in the Palaearctic, Nearctic, Neotropical and Saharo-Arabian zoogeographical regions (Zmudzinski & Skoracki, 2017). Until now, *Torotrogla* has been represented by 18 species associated with several groups of passeriform hosts (35 species...
and 14 families) (Glowska et al., 2015a, b; Skoracki et al., 2016; Zmudzinski & Skoracki, 2017). Currently, this genus is in the field of molecular research interests, because of the phenotypic plasticity observed in *T. merulae* and *T. rubeculi* (see Glowska et al., 2013) and due to its species being hosts for endosymbiotic bacteria of the genus *Wolbachia* Hertig & Burt 1924 (see Glowska et al., 2015b). All this indicates that basic systematic studies on quill mites, whenever possible, should be extended with DNA data. As it has been shown, molecular tools may be successfully used for syringophilid species descriptions (Glowska et al., 2012a, b), tests of host specificity (Glowska et al., 2016), delimitation of species boundaries (Glowska et al., 2013, 2014) and detecting cryptic species (EG, unpublished data).

In this paper, a new quill mite species *Torotrogla paenae* n. sp. parasitising the Kalahari scrub-robin *Cercotrichas paena* (Smith) (Passeriformes: Muscicapidae) in Namibia is described based on morphological and DNA barcode data. *Cercotrichas paena* is a new host species for the family Syringophilidae and the first record of the genus *Torotrogla* in the Afrotropical realm.

**Materials and methods**

**Animal material and morphological analysis**

Mite material used in the study was acquired from the collection of feathers deposited in the Smithsonian Institution, National Museum of Natural History, Department of Vertebrate Zoology, Division of Birds, Washington, DC, USA (USNM) (September 2014). Bird specimen was collected in Namibia, 2009. Drawings were made with an Olympus BH2 microscope with differential interference contrast (DIC) optics and a camera lucida. All measurements are in micrometres. The idiosomal setation follows Grandjean (1939) with modifications adapted for Prostigmata by Kethley (1990). The system of nomenclature for leg chaetotaxy follows that proposed by Grandjean (1944). The application of these chaetotaxy schemes to Syringophilidae was recently provided by Bochkov et al. (2008) with changes by Skoracki (2011). Latin and common names of the bird species follow Clements et al. (2017).

Material depositories and abbreviations: AMU, Adam Mickiewicz University, Poznan, Poland; USNM, Smithsonian Institution, National Museum of Natural History, Washington, DC, USA. The voucher slides and corresponding DNA samples are deposited in the collection of the AMU under the identification numbers as indicated below. The sequences were deposited in the GenBank database under the accession numbers MG948551 (*cox1*) and MG952940 (D1 region of the 28S rRNA gene).

**Molecular data and analysis**

Total genomic DNA was extracted from single specimens using DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) as described by Dabert et al. (2008). We used sequence data for the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene and the D1 region of the nuclear 28S rRNA gene. *cox1* was amplified by PCR with degenerate primers Aseq01F (5′-GGA ACR ATA TAY TTT ATT TTT AGA-3′) and Aseq03R (5′-GGA TCT CCW CCT CCW GAT GGA TT-3′) (Glowska et al., 2014). The D1 region of the 28S rRNA gene was amplified using the primer pair 28SF0001 (5′-ACC CVC YNA ATT TAA GCA TAT-3′) (Mironov et al., 2012) and 28SR0450 (5′-TTT GCA ACT TTC CCT CAC GG-3′) (newly designed). PCR amplifications were carried out in 10 μl reaction volumes containing 5 μl of Type-it Microsatellite Kit (Qiagen), 0.5 μM of each primer, and 4 μl of DNA template using a thermocycling profile of one cycle of 5 min at 95°C followed by 35 cycles of 30 s at 95°C, 1 min at 50°C, 1 min at 72°C, with a final step of 5 min at 72°C. After amplification PCR products were two-fold diluted with water, and 5 μl of the sample was analyzed by electrophoresis on a 1.0% agarose gel. Samples containing visible bands were purified with thermostensitive Exonuclease I and FastAP Alkaline Phosphatase (Fermentas, Thermo Scientific, Göteborg, Sweden). The amplicons were sequenced in one direction using PCR forward primers. Sequencing was performed with BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence chromatograms were checked for accuracy and edited using FinchTV 1.3.1 (Geospiza, Inc.) and manually aligned in GeneDoc v.2.7.000 (Nicholas & Nicholas, 1997).
Family Syringophilidae Lavoipierre, 1953
Subfamily Syringophilinae Lavoipierre, 1953
Genus Torotrogla Kethley, 1970

Torotrogla paenae n. sp.

Type-host: Cercotrichas paena (Smith) (Passeriformes: Muscicapidae), Kalahari scrub-robin.

Type-locality: Omahewe, Aminuis (23°51'01"S, 19°33'42"E), Namibia.

Type-material: Holotype female, 2 female and 1 male paratypes (USNM 642341) are deposited in the USNM, 2 female paratypes in the AMU; coll. E. Gowska (10.viii.2009).

Representative sequences: GenBank accession numbers for molecular voucher code KR003: MG948551 (cox1) and MG952940 (D1 of the 28S rRNA gene).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for Torotrogla paenae n. sp. is urn:lsid:zoobank.org:act:0F60B0D1-6A08-4C04-8680-6B3BF2C6C295.

Etymology: The name is after the specific name of the host.

Description (Figs. 1–10)

Female [Based on the holotype and 4 paratypes (data in parentheses); Figs. 1–5.] Total body length 800 (780–830 in 4 paratypes). Gnathosoma. Hypostomal apex with pair long, sharp-ended protuberances (Fig. 3). Each medial branch of peritremes with 4 chambers, each lateral branch with 4–6 chambers (Fig. 4). Stylophore constricted posteriorly, 215 (215) long. Idiosoma. Propodonotal shield apunctate. Agenital series represented by a pair of ovate plates bearing bases of setae d1. Length ratio of setae d2:d1:e2 1:1.1:1.4:1–1.5. Pygidial shield well sclerotised and apunctate. Agenital series represented by 5–7 setae on each body side. Legs. All coxal fields apunctate. Setae tc' and tc'' of legs III–IV subequal in length. Fan-like tarsal setae p' and p'' of legs III–IV with c.10 tines (Fig. 5). Lengths of setae: vi 80 (70); ve 130 (105–120); si 140 (160–180); se 185 (160–185); c2 195 (195–215); c1 230 (215–245); d1 170 (155–185); d2 140 (130–150); e2 195 (155–200); f1 55 (75–90); f2 325 (305–370); h1 65 (65–70); h2 405 (345–375); ps1 and ps2 35 (35–40); g1 and g2 40 (45–55); 3b 30 (50–60); 3c 80 (70–90); 4b 45 (30–60); 4c 70 (60–105); tc”III–IV 70 (65–70); tc”III–IV 60 (65–75); l’RIII 40 (55–65); l’RIV 45 (35–45).

Male [Based on 1 paratype; Figs. 6–10.] Total body length 555. Gnathosoma. Hypostomal apex with pair long, sharp-ended protuberances. Each medial branch of peritremes with 4–5 chambers, each lateral branch with 4 chambers (Fig. 9). Stylophore constricted posteriorly, 165 long. Idiosoma. Propodonotal shield apunctate, rectangular in shape, bearing bases of setae vi, ve, si and cl. Length ratio of setae vi:ve:si 1:1.1:3. Setae se situated anterior to level of setae cl. Hysteronotal shield bearing bases of setae d1, e2, f2 and h2. Length ratio of setae d2:d1:e2 1.5:1.5. Setae h2 5.8 times longer than f2. Agenital series represented by 3 setae on each body side. Setae g1,2 and ps1,2 subequal in length. Legs. All coxal fields apunctate. Setae tc’ and tc” of legs III–IV subequal in length. Fan–like setae p’ and p” of legs III–IV with 8 tines. Lengths of setae: vi 40; ve 45; si 120; se 120; c2 120; c1 120; d1 20; d2 30; e2 30; f2 35; h2 205; ps1,2 10; g1,2 10; 3b 30; 3c 60; 4b 45; 4c 45; tc”III–IV 45; tc”III–IV 45; l’RIII 30; l’RIV 20.

DNA barcodes

The cox1 sequence data were generated from three females of Torotrogla paenae n. sp. All specimens shared the same cox1 haplotype (GenBank: MG948551). For one specimen a 305-bp fragment coding for the region D1 of 28S rRNA gene was sequenced as DNA-barcode for nuclear DNA (GenBank: MG952940).

Remarks

This new species Torotrogla paenae n. sp. is morphologically most similar to T. lusciniae Skoracki, 2004 described from the common nightingale Luscinia megarhynchos Brehm (Muscicapidae) from Italy (Skoracki, 2004). Females of both species have the hypostomal apex provided with a pair of long sharp-
Figs. 1–5  *Torotrogla paenae* n. sp., female: 1, Dorsal view; 2, Ventral view; 3, Hypostomal apex; 4, Peritremes; 5, Fan-like setae *p’ of leg III. Scale-bars: 1, 2, 200 μm; 3–5, 50 μm

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Figs. 6–10  Torotrogla paenae n. sp., male: 6, Dorsal view; 7, Ventral view; 8, Hypostomal apex; 9, Peritremes; 10, Fan-like setae p' of leg III. Scale-bars: 6–7, 200 μm; 8–10, 30 μm
ended protuberances, idiosomal setae \( f1 \) and \( h1 \) short and subequal in length, and setae \( tc' \) and \( tc'' \) of legs III–IV subequal in length. Females of \( T. \) paenae n. sp. differ from \( T. \) lusciniae by the following characters: the total body length is 780–830 \( \mu \)m (vs 645–715 \( \mu \)m in \( T. \) lusciniae), the hysteronotal shields are present (vs absent), the propodonotal and pygidial shields are apuncate (vs punctate), all coxal fields are apuncate (vs punctate), the fan-like tarsal setae \( p' \) and \( p'' \) of legs III–IV are provided with c.10 tines (vs 14–15) and the length of setae \( si \) (140–180 vs 190–210 \( \mu \)m) and \( se \) (160–185 vs 210–225 \( \mu \)m). Males of \( T. \) paenae n. sp. differ from \( T. \) lusciniae in having the lateral branch of peritremes composed of 4 chambers (vs 7–8 chambers), and lengths of setae \( ve \) (45 vs 70–75 \( \mu \)m) and \( se \) (120 vs 165 \( \mu \)m).

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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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**References**

Bochkov, A. V., OConnor, B. M., & Wauthy, G. (2008). Phylogenetic position of the family Myobiidae within the Prostigmata (Acari: Acariformes). Zoologischer Anzeiger, 247, 15–45.

Casta, S. D. (1976). Host records and observations of quill mites (Acarina: Syringophilidae) from Texas birds. Southwestern Entomologist, 1, 155–160.

Clements, J. F., Schultenberg, T. S., Liiff, M. J., Roberson, D., Fredericks, T. A., Sullivan, B. L., & Wood, C. L. (2017). The eBird/Clements checklist of birds of the world: v2017. http://www.birds.cornell.edu/clementschecklist/download/. Accessed 15 May 2018.

Dabert, J., Ehrnsberger, R., & Dabert, M. (2008). Glaucaleges tytonis sp. n. (Acanthoidea, Xolalgiidae) from the barn owl Tyto alba (Strigiformes, Tytonidae): Comparing morphological data with DNA barcode data for taxon descriptions in mites (Acari). Zootaxa, 1719, 41–52.

Glowska, E., Chrzansowski, M., & Kaszewska, K. (2015a). Checklist of the quill mites (Acariformes: Syringophilidae) of the World. Zootaxa, 3968, 1–81.

Glowska, E., Dragun-Damian, A., & Dabert, J. (2012a). Picobia dziabaszewskii sp. nov. (Acani, Syringophilidae) - Combined description (morphology with DNA barcode data) of a new quill mite species parasitizing Garrulax formosus (Passeriformes: Leiothrichidae). Zootaxa, 3224, 57–61.

Glowska, E., Dragun-Damian, A., & Dabert, J. (2012b). A new quill mite Syringophiloidus pseudonigrigerae sp. nov. (Prostigmata, Syringophilidae) parasitizing Pseudonigrigrita arnauti (Passeriformes, Ploceidae) - a combined description using morphology and DNA barcode data. Zootaxa, 3532, 64–68.

Glowska, E., Dragun-Damian, A., & Dabert, J. (2013). DNA-barcoding contradicts morphology in quill mite species Torotroga merulae and T. rubeculi (Prostigmata: Syringophilidae). Folia Parasitologica, 60, 51–60.

Glowska, E., Dragun-Damian, A., Broda, L., Dabert, J., & Dabert, M. (2014). DNA barcodes reveal female dimorphism in syringophilid mites (Acnitrichida: Prostigmata: Cheyletoidae): Stibanorakis phoeniconaias and Cicconichenophilus phoeniconaias are conspecific. Folia Parasitologica, 61, 272–276.

Glowska, E., Dragun-Damian, A., Dabert, M., & Gerth, M. (2015b). New Wolbachia supergroups detected in quill mites (Acari: Syringophilidae). Infection, Genetics and Evolution, 30, 140–146.

Glowska, E., Broda, L., Gebhard, Ch A, & Dabert, M. (2016). A new quill mite Syringophiloidus plocei sp. nov. (Prostigmata: Syringophilidae) parasitizing ploceid birds (Passeriformes) in Gabon - A combined description using morphology and DNA barcoding. Acta Parasitologica, 61, 562–566.

Grandjean, F. (1939). Les segments postlarvaires de l’hysterosoma chez les oribates (Acariens). Bulletin de la Société Zoologique de France, 64, 273–284.

Grandjean, F. (1944). Observations sur les acariens de la famille Stigmaeidae. Archives des Sciences Physiques et Naturelles, 26, 103–131.

ICZN (2012). International Commission on Zoological Nomenclature: Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. Bulletin of Zoological Nomenclature, 69, 161–169.

Kethley, J. B. (1990). Acarina: Prostigmata (Actinedida). In: Dindal, D. L. (Ed.), Soil Biology Guide. New York: Wiley, pp. 667–754.

Mironov, S. V., Dabert, J., & Dabert, M. (2012). A new feather mite species of the genus Proctophyllodes Robin, 1877 (Astigmata, Proctophyllodidae) from the long-tailed tit Aegithalos caudatus (Passeriformes: Aegithalidae) -
Morphological description with DNA barcode data. Zoo-taxa, 3253, 54–61.
Nicholas, K. B., & Nicholas, Jr. H. B. (1997). GeneDoc: a tool for editing and annotating multiple sequence alignments. Pittsburgh Supercomputing Center’s National Resource for Biomedical Supercomputing, ver. 2.7.000. http://www.nrbsc.org/downloads. Accessed 2 May 2018.
Skoracki, M. (2004). New data on systematics of the quill mites of the Torotrogla Kethley, 1970 (Acari, Syringophilidae). Belgian Journal of Entomology, 6, 303–314.
Skoracki, M. (2011). Quill mites (Acari: Syringophilidae) of the Palaearctic region. Zootaxa, 2840, 1–414.
Skoracki, M., Spicer, G. S., & O’Connor, B. M. (2016). A systematic review of the subfamily Syringophilinae (Acari: Syringophilidae) of the Nearctic region. Part 1: Quill mites associated with passerines (Aves: Passeriformes). Zootaxa, 4084, 451–494.
Zmudzinski, M., & Skoracki, M. (2017). An updated check-list of the family Syringophilidae (Acariformes: Prostigmata). Available at https://sites.google.com/site/syringophilidae/. Accessed 15 May 2018.