First record of *Streptosyllis nunezi* Faulwetter et al., 2008 (Annelida, Syllidae) from the United Kingdom, and amendment to the genus *Streptosyllis* Webster & Benedict, 1884

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Abstract

During a benthic survey of a Marine Conservation Zone located on the Skerries Bank in the English Channel off the south-west coast of England, three specimens of *Streptosyllis nunezi* Faulwetter et al., 2008 (Annelida, Syllidae) from the United Kingdom, and amendment to the genus *Streptosyllis* Webster & Benedict, 1884 were found. This is the second ever record of the species after its original description, and the first record from waters around the U.K. and a significant northerly range extension for a species previously only recorded from the Canary Islands and the Mediterranean Sea. A single simple ventral chaeta in each of the two posterior-most segments was discovered in this and two other species of *Streptosyllis* Webster & Benedict, 1884. The generic definition of *Streptosyllis* is emended to include this feature previously unknown for the genus, and an updated key to the *Streptosyllis* found in UK waters is provided.

Keywords

Polychaetes, simple ventral chaeta, generic amendment, identification key
Introduction

Species of the genus *Streptosyllis* Webster & Benedict, 1884 are small-sized polychaetes living interstitially in shallow marine sediments of sand, muddy sand or sandy mud (San Martín 2003). The genus currently comprises 16 species (Gil and Read 2015) and is characterised by an unarmed pharynx, palps fused at the base, enlarged knob-tipped aciculae and modified compound chaetae in anterior segments (Brito et al. 2000, San Martín 2003). To date, five species of the genus have been reported from UK waters, or waters adjacent to the UK (Govaere et al. 1980, Howson and Picton 1997, San Martín and Worsfold 2015). Three of these species were originally described from Europe: *S. bidentata* Southern, 1914 from Ballynakill, Ireland, *S. campoyi* Brito, Núñez & San Martín, 2000 from the Canary Islands, Spain, and *S. websteri* Southern, 1914 from Bofin, Ireland. The two other species, rarely reported from the UK, were originally described from the east coast of the USA: *S. arenae* Webster & Benedict, 1884 from Provincetown, Massachusetts and *S. varians* Webster & Benedict, 1887 from Eastport, Maine. The records for the latter two species however are questionable. Hartmann-Schröder (1996) considered the record of *S. arenae* to be suspect, while Saint-Joseph’s (1895) description of *S. varians* from Dinard (repeated in Fauvel 1923) does not fit the original description of Webster and Benedict (1887) and is believed to belong to a different species of the genus (Southern 1914).

In this paper, *Streptosyllis nunezi* is reported from UK waters for the first time and is the second record of the species after its original description. Furthermore, additional material of *S. websteri* from both the UK and from Greece, and of *S. campoyi* from the UK was examined and a previously unreported chaetal type was found in all three species of the genus. The generic definition of *Streptosyllis* is emended accordingly below.

Methods

The Skerries Bank and Surrounds candidate Marine Conservation Zone (MCZ), situated off the south-west coast of England, was designated in November 2013 as part of the designation of 27 sites (Marine and Coastal Access Act 2009). In order to verify the species and habitats present, the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in collaboration with the Environment Agency (EA) undertook a benthic survey of the Skerries Bank and Surrounds site in January 2014. The benthos was sampled using a 0.1 m² Hamon grab, sieved through a 1.0 mm mesh and fixed in a buffered 4% formaldehyde seawater solution.

Specimens belonging to the genus *Streptosyllis* were found in the samples collected at sites GT193 (50.2537, -3.6058; depth 13.4 m) and GT204 (50.2429, -3.6110, depth 9.2 m) (Fig. 1). Both sites are located approximately 2.1 miles (3.5 km) from the coast, off Beesands in Devon, and are characterised by coarse to medium sands. The only other taxa common to both samples were *Nephtys cirrosa* Ehlers, 1868 and Nemertea species.
In addition, several *Streptosyllis* specimens in archive material of the Institute of Estuarine and Coastal Studies’ (IECS) were examined, which were collected during a benthic survey carried out by Natural England (NE) around the Scilly Isles in April 2009. *Streptosyllis* specimens were found at sites SC2.5 (49.9469, -6.2846; depth around 5–8 m), Sc9.3 (49.9078, -6.3436; depth 14.5 m), Sc9.7 (49.9042, -6.3401; depth 13.2 m) and Sc9.10E (49.9064, -6.3413; depth 11.8 m) from St. Mary’s Sound, Scilly Isles (Fig. 1), from coarse to medium sands. Samples were sieved through a 0.5 mm and 1.0 mm sieve; all specimens studied here were retained by the 0.5 mm fraction. Specimens were fixed and preserved in 70% Industrial Methylated Spirits (IMS).
Samples were analysed in the laboratory by IECS, following national quality assurance guidelines for both faunal extraction and taxonomic identifications.

Additional specimens of *Streptosyllis campoyi* and *Streptosyllis websteri* from the IECS and of *S. websteri* from archive collections of the Hellenic Centre for Marine Research (HCMR) were studied for comparison; sampling details of the latter can be found in Papageorgiou et al. (2006).

**Taxonomic results**

During the identification phase of the analysis, three specimens of a *Streptosyllis* species with a distinctive hyaline hood on the blade and distal end of the shaft of the compound chaetae and serrated simple dorsal chaetae with a hyaline hood were found in samples GT193 and GT204. A further six individuals with the same characteristics were discovered in the IECS archive material in samples Sc9.3, Sc9.7 and Sc9.10E. The chaetae found in the specimens were unlike those of any of the *Streptosyllis* species confirmed so far from UK waters: *S. websteri* Southern, 1914, *S. bidentata* Southern, 1914 and *S. campoyi* Brito, Núñez & San Martín, 2000 (San Martín and Worsfold 2015). After comparison with the literature and personal notes of the second author, the specimens were confirmed to belong to *Streptosyllis nunezi*, a species so far only known from Crete, Tuscany and the Canary Islands. The examination of the specimen also revealed a single simple ventral chaeta in each parapod of the last two fully formed posterior chaetigers, a character previously thought to be absent in the genus *Streptosyllis* (San Martín 2003, San Martín and Hutchings 2006). Upon examination, specimens of *S. campoyi* and *S. websteri* from the IECS reference collection and archived material, as well as archived material of *S. websteri* from Crete were found to also possess a simple ventral chaetae in the last one or two fully formed posterior chaetigers. Based on this newly discovered character, the generic diagnosis for *Streptosyllis* is emended below.

**Genus Streptosyllis Webster & Benedict, 1884, emended**

*Streptosyllis* Webster & Benedict, 1884: 711  
*Streptosyllis* – San Martín 2003: 120  
*Streptosyllis* – San Martín and Hutchings 2006: 354  
*Streptosyllis* – Faulwetter et al. 2008: 2

**Type species.** *Streptosyllis arenæ* Webster & Benedict, 1884.

**Diagnosis.** Body small. Four eyes, occasionally anterior pair of eyespots present. Palps fused at base, occasionally reduced to small papillae. Anterior parapodia with modified compound chaetae; sometimes with enlarged aciculae. Dorsal simple chaetae present, simple ventral capillary chaetae may be present in posteriormost chaetigers.
Compound chaetae homogomph or hemigomph, falcigerous or spinigerous. Dorsal cirri smooth, pseudoarticulated or articulated with granular inclusions. Ventral cirri digitiform, sometimes longer than parapodial lobe. Pharynx unarmed with crown of soft papillae. Pygidium with one median and two lateral anal cirri.

Streptosyllis nunezi Faulwetter, Vasileidadou, Papageorgiou & Arvanitidis, 2008


Material examined. 3 individuals from the Skerries Bank, England, 9–13 m depth; 6 individuals from the Scilly Isles, England, 11–14 m depth, at both sites in coarse to medium sand.

Description. Body ca. 5 mm long, for 64 chaetigers in the only complete animal. Head semi-circular with two pairs of eyes and two eyespots located anteriorly. Three smooth antennae, median one twice as long as lateral ones. Palps basally fused, forming two rounded lobes, not visible dorsally. Two pairs of smooth tentacular cirri, about as long as lateral antennae. Dorsal cirri about as long as or slightly shorter than body width, smooth anteriorly, after proventricular region at irregular distances with pseudo-articulations containing yellow granular inclusions. Ventral cirri digitiform, smooth, almost as long as parapodial lobes anteriorly and in midbody, longer than parapodial lobe posteriorly. Posteriormost 3 segments achaetous. Pygidium with single filiform ventral cirrus (2 lateral cirri missing?). Up to 8 compound falcigers in each parapodium. Shafts of compound chaetae with three hemigomph teeth, sometimes notched so that they appear as up to four teeth (Fig. 2A–B). Blades of falcigers of two types: short ones (ca. 7–9 µm) and longer ones (ca. 15 µm), former ones occurring in anteriormost chaetigers, longer ones in two dorsalmost chaetae of midbody and in posterior chaetigers (Fig. 2C–E). Short blades covered entirely by membrane forming blunt tip and notch alongside of blade; longer blades covered by membrane forming pointed tip if viewed laterally, blunt if viewed from top, and 1–2 teeth along cutting edge of blade (Fig. 2C–D). Membrane of blades often extending to shaft, covering its top. Posteriorly, all blades of compound chaetae thin and elongated (Fig. 2E). One dorsal simple chaeta present per chaetiger, from anteriormost chaetigers, slightly curved, tip bluntly rounded, covered by membrane forming blunt tip. Strong serration on distal end just below hood, forming up to 4 large, round teeth (Fig. 2F). One ventral simple chaeta in each of two last posteriormost chaetigers (excluding developing ones), very thin, capillary-like (Fig. 2G). Single acicula per parapodium, distally knobbed, knob sometimes irregularly rounded with one side longer than the other, anteriorly sometimes protruding from parapodium. Aciculae slightly enlarged in segments 3, 4, and 5, about 1.5–2 times larger than those of preceding and proceeding chaetigers (Fig. 2H–I). Pharynx through 3–5 segments, proventricle through 6 segments.
Figure 2. *Streptosyllis nunezi*, A anterior parapodium with falcigers with short blades B parapodium, mid-body C, D falcigers with elongated blade, mid-body; E falciger with elongated blade, posterior chaetiger F dorsal simple chaeta G posterior parapodium with ventral simple chaeta (arrow) H aciculae of chaetigers 2–6 (numbered) I acicula, mid-body. Scale bars: 10 µm (A–G, I), 20 µm (H).

Remarks. Except for the presence of ventral chaetae and the aciculae protruding from the parapodium, all examined animals correspond well to the original description by Faulwetter et al. (2008).
First record of Streptosyllis nunezi Faulwetter et al., 2008 (Annelida, Syllidae)...

**Distribution.** Mediterranean Sea (Crete, Italy), northeastern Atlantic (Canary Islands, Scilly Islands, Skerries Bank)

**Ecology.** Occurs in fine to coarse sandy substrates in shallow waters (1–20 m).

**Streptosyllis campoyi** Brito, Núñez & San Martín, 2000

*Streptosyllis campoyi* Brito, Núñez & San Martín, 2000: 611, figs 5a–l.
*Streptosyllis bidentata* (non Southern) – Campoy 1982: 314, figs 25 a–j.

**Material examined.** 3 individuals from Station Sc9.7, St. Mary’s Sound, Scilly Isles, 13.2 m depth, in coarse to medium sand.

**Remarks.** All examined animals correspond well to the description provided by San Martín (2003), except for the presence of a thin, capillary-like ventral simple chaeta in each of the two posteriormost chaetigers (Fig. 3A).

**Streptosyllis websteri** Southern, 1914

*Streptosyllis websteri* Southern, 1914: 26, pl. II, figs 3 a–f.
*Streptosyllis websteri* – Hartmann-Schröder 1996: 163, fig. 69; San Martín 2003: 125, figs 59–60; San Martín and Aguado 2006: 731, fig. 2; Nygren and Pleijel 2015: 182.

**Material examined.** 15 individuals from Elafonisi Island, Crete, 0–1 m depth; 1 individual from Pahia Ammos, Crete (5 m depth), at both sites in coarse sand; 2 individuals from Station Sc2.5 Scilly Isles, depth around 5–8 m.

**Remarks.** All examined animals correspond well to the description provided by San Martín (2003), except for the presence of a single thin, capillary-like ventral simple chaeta in each of the two posteriormost chaetigers (Fig. 3B).

![Figure 3](image-url). Posterior parapodium with ventral simple chaeta (arrow) of **A** *Streptosyllis campoyi** B *Streptosyllis websteri*. Scale bars: 10 µm
Discussion

*Streptosyllis arenae* is morphologically the most similar species to *Streptosyllis nunezi*; it differs from *S. arenae* by the presence of the hyaline hood covering the distal end of the shaft of the compound chaetae, and the rounded teeth found on the shaft of the dorsal simple chaetae. Other *Streptosyllis* species which have a hyaline hood on the blades of their compound chaetae are *S. biarticulata* Hartmann-Schröder, 1991, and *S. magnapalpa* Hartmann-Schröder, 1981. *Streptosyllis nunezi* can also be distinguished from both these species again by the presence of the hyaline hood covering the distal end of the shaft of the compound chaetae, and the rounded teeth found on the shaft of the dorsal simple chaetae (Faulwetter et al. 2008); furthermore these species are only known so far from Australia. *Streptospinigera templadoi* (San Martín, 1984) also has hyaline hood-like structures on its compound chaetae, however it also lacks the hyaline hood on the distal end of the shaft of the compound chaetae and teeth on the dorsal simple chaetae, furthermore it possesses spinoigerous compound chaetae not seen in *S. nunezi* (Olivier et al. 2013).

*Streptosyllis arenae* was recorded by Govaere et al. (1980) and Vanosmael et al. (1982) from the southern North Sea, but whether these records really pertain to *S. arenae*, originally described from Provincetown, Massachusetts, USA, is unknown, though Hartmann-Schröder (1996) suspected them to be *S. websteri*. These records might in fact also belong to *S. nunezi*, which is here shown to have a more northerly distribution than previously known, but investigation of the material would be needed to confirm this. Recently, *S. nunezi* has been recorded by one of us (WM) from the eastern Humber region of the North Sea. This could lend additional support to the hypothesis that previous records of *S. arenae* from the North Sea could be referred to *S. nunezi* and that the species might be native to the region. Its small size and the fact that only recently appropriate keys for the group have been published might have contributed to it being overlooked or misidentified in the past. An updated key to the UK species of the genus is provided below.

Key to the *Streptosyllis* (Webster & Benedict, 1884) species found in UK waters
(adapted from San Martín and Worsfold (2015))

1. Compound chaetae with hyaline hood-like structures around the blade and distal end of the shaft and a strong serration on the distal end of the simple dorsal chaetae........................................... *S. nunezi* Faulwetter et al., 2008
   - Compound chaetae without hyaline hood-like structures...........................2
2. Compound chaetae with indistinctly bidentate blades. Strongly enlarged aciculae in chaetigers 2–5........................................... *S. websteri* Southern, 1914
   - Compound chaetae with distinctly bidentate blades. Strongly enlarged aciculae in chaetigers 2–6............................................................................................3
Blades of compound chaetae with both teeth similar and close to each other. Aciculae of chaetiger 7 only slightly more slender than those of chaetiger 6.... 

S. bidentata Southern, 1914

Blades of compound chaetae with proximal teeth longer and well separated. Aciculae of chaetiger 7 distinctly more slender than those of chaetiger 6....... 

S. campoyi Brito, Núñez & San Martín, 2000

Conclusions

In conclusion, the findings of this study showed a significant northerly range extension for Streptosyllis nunezi previously only confirmed from the Canary Islands and the Mediterranean Sea. A single simple ventral chaetae in each of the two posteriormost segments was also discovered in this and two other species of Streptosyllis, resulting in an emended diagnosis of the genus.

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References


Systematics of testudacarine torrent mites (Acari, Hydrachnidia, Torrenticolidae) with descriptions of 13 new species from North America

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Abstract

Thirteen new species of North American Testudacarus (Torrenticolidae: Testudacarinae) are described: T. deceptivus O’Neill & Dowling, sp. n., T. hitchensi O’Neill & Dowling, sp. n., T. harrisi O’Neill & Dowling, sp. n., T. dennetti O’Neill & Dowling, sp. n., T. dawkinsi O’Neill & Dowling, sp. n., T. radwellae O’Neill & Dowling, sp. n., T. kirkwoodae O’Neill & Dowling, sp. n., T. hyporhynchus O’Neill & Dowling, sp. n., T. smithi O’Neill & Dowling, sp. n., T. rolleræ O’Neill & Dowling, sp. n., T. elongatus O’Neill & Dowling, sp. n., T. rectangulatus O’Neill & Dowling, sp. n., and T. oblongatus O’Neill & Dowling, sp. n. Testudacarus vulgaris Habeeb, 1954 is resurrected from synonymy with T. minimus and redescribed. Debsacarus (Habeeb, 1961), Testudacarus americanus Marshall, 1943, and T. minimus Marshall, 1943 are redescribed. All redescriptions are from original types. Species delimitation was accomplished through examination of morphology, biogeography, and molecular phylogenetics of the barcoding region of COI. Other species are addressed and a key to world species is presented. For Testudacarinae, this represents the first published: 1) descriptions from multiple specimens (i.e. intraspecific variation); 2) colored photographs; 3) explicit illustrations and discussion of sexual dimorphism within the subfamily; 4) genetic data. A comprehensive testudacarine reference list is also included.

Keywords

Hydrachnidiae, Hydrachnidia, water mites, Testudacarinae, Testudacarus, Debsacarus

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Introduction

Torrenticolidae Piersig, 1902 are ubiquitous and diverse in North America, but the majority of species remain undescribed. This study is the second in a series of descriptions of North American torrenticolids. The goal of this ongoing taxonomic project is to explore the family and make these mites amenable to other researchers.

Testudacarinae Cook, 1974 are found abundantly in riffles of fast flowing streams throughout most of North America and sporadically in Asia. Typical of lotic-dwelling water mites, testudacarines are dorso-ventrally flattened, heavily sclerotized, and possess robust legs with large tarsal claws used for crawling. Most testudacarines are less than 1 mm in size and can exhibit striking coloration. Larvae are reported to be ectoparasites of chironomid adults (Smith 1982).

Despite their abundance, few testudacarines are described worldwide and in North America the most recent description is over fifty years old. Limited morphological and distributional data have been presented, and no genetic data has ever been published on Testudacarinae. Minimalistic and incomplete descriptions have led to considerable confusion throughout testudacarine taxonomic history. There is a need to describe new species with modern methods and to redescribe older species with the same thoroughness.

Thirteen descriptions and four redescriptions of North American Testudacarus Walter, 1928 are included within. Following Fisher et al. (2015), species were delimited using a combination of morphology, biogeography, and molecular data (i.e. “barcoding” region of COI). In addition to descriptions and redescriptions, sexual dimorphism within the subfamily is explicitly addressed, a comprehensive testudacarine reference list is included, and a key to world species is presented.

Taxonomic history

There are currently nine testudacarines described worldwide: Testudacarus tripeltatus Walter, 1928 from India; T. japonicus Imamura, 1955 and T. okadai Imamura, 1976 from Japan; T. binodipalpis Guo and Jin, 2005 from China; and T. americanus Marshall, 1943, T. minimus Marshall, 1943, T. minimus vulgaris (Habeeb, 1954), T. americanus galloi Habeeb, 1969, and Debsacarus oribatoides (Habeeb, 1961) from the United States. However, the status of several of these testudacarines remains unclear.

Testudacarus americanus and T. minimus were described by Marshall (1943) from one “small” male and one “large” female from the same creek in California. Habeeb (1954) described T. vulgaris from New Brunswick. Later, Habeeb (1967) synonymized T. minimus with T. americanus after noticing sexual dimorphism within Testudacarus (specifically, females are larger than males). Habeeb (1969) then synonymized T. vulgaris with T. americanus and established Testudacarus americanus galloi, from “two female mites rather like [T. americanus], yet atypical.” He stated
that *T. americanus vulgaris* was a blue form found from New Brunswick to as far west as Arizona, and *T. americanus americanus* and *T. americanus minimus* were “red to golden” forms found from California. Habeeb (1974a) then resurrected *T. minimus* and changed *T. americanus vulgaris* to *T. minimus vulgaris*, after realizing he had misread Marshall (1943).

Habeeb (1961) described *T. oribatoides* from a male and female from California. This species has a “protrusable maxillary tube…reminiscent of *Pseudotorrenticola,*” and is in other respects atypical for *Testudacarus* (Habeeb 1961). Habeeb (1974b) erected *Debsacarus* and designated *Testudacarus oribatoides* as the type specimen “due to the fact that many recent authors have no respect for subgeneric names.”


**Methods**

**Sampling and curation**

Mites were collected and preserved using protocols detailed in Fisher et al. (2015).

**Morphological terminology**

Terminology used in this study is detailed in Figs 1–5 and follows Goldschmidt (2007) as modified by Fisher et al. (2015). Hyphens are used for directional or numbered morphological features: for example, dorsoglandularia 1 will be expressed as dorsoglandularia-1. This is to prevent confusion when terms are followed by numbers and to make longer, more complicated terminology more accessible to unfamiliar readers. “Colorless” refers to a lack of pigmentation in the cuticle; as the cuticle itself is typically yellowish, “colorless” species are thus yellowish.

**Images and measurements**

Images were produced and measurements taken following the protocol detailed in Fisher et al. (2015). Measurements follow Goldschmidt (2007) with additions.
Figure 1. Testudacarine male dorsum (generalized): (Left) – antero-medial platelet (amp); anterio-lateral platelet (alp); dorsal plate (dp); lateral platelets (lp); (Right) – dorso-glandularia (dg); post-ocularial setae (pos); dorsal membrane (dm); lyrifissures (l); muscle scars (ms); latero-glandularia (lg).

Material deposition of Nearctic types

All holotypes, allotypes, and some paratypes have been deposited in the Canadian National Collection of Insects, Arachnids, and Nematodes (CNC), Ottawa, Canada. Additional paratypes have been deposited in the Acari Collection of the University of Arkansas (ACUA), Fayetteville, Arkansas. Specific numbers of slides deposited at the CNC and ACUA are noted within each species description. Collection abbreviations are used throughout.
Systematics of testudacarine torrent mites (Acari, Hydrachnidia, Torrenticolidae)...

Figure 2. Testudacarine male dorsum (SEM): antero-medial platelet (amp); antero-lateral platelet (alp); dorsal plate (dp); dorso-glandularia (dg); post-ocularial setae (pos); dorsal membrane (dm); latero-glandularia (lg). Scale: 100 µm. Photo Michelle Hoppner and Ian Smith (used with permission).

Morphological and distributional examinations

Material from the CNC and additional collections provided tens of thousands of testudacarines for morphological examination from across North America; a portion of these were examined closely for morphological variation. Previous torrenticolid studies suggested color and size were not necessarily important characters in distinguishing species (e.g., Fisher et al. 2015). Therefore, testudacarine “morphotypes” were chosen conservatively, giving more weight to drastic character differences, such as the presence of four instead of five pedipalp segments, over potentially more ambiguous characters, such as color and size variation. Many morphological characters were examined including general characteristics (e.g., color, size, body shape) and specific morphological features such as the following: shape of the dorsal plate, platelets, coxal field, and genital field; positioning of glandularia and lyrifissures; setae on the dorsum, venter, and gnathosoma; and structure of the gnathosoma and ejaculatory complex. Over 100 measurements per specimen were taken and compared and proportions between many of these measurements were analyzed. Finally, distributional
Figure 3. Testudacarine male venter (generalized): **Left** – coxo-glandularia (cg); latero-glandularia (lg); ventro-glandularia (vg); **Middle** – coxae (c). **Right** – gnathosomal bay (gb); coxae-II+III midline (ml); genital field (gf); acetabula (a); line of secondary sclerotization (ss); excretory pore (ep).

data was considered for each “morphotype” and probable ranges were hypothesized. Differences and similarities in ranges were considered as further supporting evidence of putative species.
Molecular examination

The “barcoding” region of COI was used as an independent test of morphological species hypotheses. COI was used to determine if any morphological characters, conservative or ambiguous, indicated species boundaries by sorting into distinct genetic lineages. COI was also used in the same way to test distributional hypotheses. Taxon sampling included roughly 300 specimens spanning “morphotypes” from across North America. Unfortunately, ethanol collections were limited from Mexico, northern Canada, and the eastern United States and therefore do not fully represent the ranges of species from these regions. Later, twenty specimens were included for phylogenetic analysis of 28S (D1-3) to investigate interspecific relationships. Genbank accession numbers of specimens for which sequences were obtained and used in this study are located in Table 1. Based upon recommendations by Chakrabarty et al. (2013), GenSeq nomenclature is used in the table to indicate the status of types and non-types sequenced.
Table 1. Genbank accession numbers and GenSeq nomenclature for each specimen sequenced for this study.

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Figure 5. Testudacarine gnathosoma (generalized): chelicerae (c); posterio-dorsal apodeme (pda); posterio-ventral apodeme (pva); subcapitulum (sc); fang (f); rostrum (r).
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Genomic DNA extraction was completed with Qiagen DNeasy Tissue Kits (Qiagen Inc., Valencia, California). Amplifications of the target region of COI were performed with LCO1490 and HCO2198 (Folmer et al. 1994). Amplifications of the target region of 28S were performed with D23F and D6R (Park and Ó Foighill 2000). PCR was performed in a DNA Engine Peltier thermal cycler. COI samples were denatured for two minutes at 94 °C, followed by forty cycles of fifty seconds
at 94 °C, thirty seconds at 48 °C, and one minute at 72 °C, with a final ten minute extension on the last cycle. 28S samples were denatured for two minutes and thirty seconds at 94 °C, followed by forty cycles of thirty seconds at 94 °C, twenty seconds at 53 °C, and one minute at 72 °C, with a final ten minute extension on the last cycle. Purification was done with Qiagen QIAquick PCR Purification Kits and test gels of 1.5% agarose were used to confirm PCR product quality. The purified product was then sequenced by Macrogen USA, based in Rockville, Maryland (http://macrogenusa.com/). DNASTAR® Lasergene SeqMan (Madison, Wisconsin) was used to reconcile forward and reverse sequences. The contigs that resulted were examined for contamination with GenBank BLAST searches. Clustal X (Thompson et al. 1997) was used to align sequences, and then BioEdit (Hall 1999) was used to conservatively edit the resulting sequences. COI sequences were around 650bp and 28S sequences were around 800bp. MrBayes (3.2.2) was used to perform Bayesian analyses over 5 million generations with Lebertia Neuman, 1880 as an outgroup. Monophyly was tested across Torrenticolidae as part of a forthcoming study. Molecular analysis was performed with the Extreme Science and Engineering Discovery Environment infrastructure available through the Cipres Portal (Miller et al. 2010).

**Species delimitation results**

Phylogenetic analysis of COI and 28S resulted in five well-supported (posterior probability greater than 95%) clades; however, analyses did not produce resolution at the base of Testudacarinae, resulting in a five-branched polytomy (Fig. 6). Each of the five lineages show at least 15% COI divergence from another. Within these five lineages are 16 distinct and well-supported species. With few exceptions, these species exhibited relatively high COI divergence (greater than 5%) between clades and relatively low divergence within a given clade (less than 1.5%). Genetic extractions were unsuccessful for a 17th species, *T. radwellae*.

Three morphotypes (*Testudacarus minimus*, *T. hitchensi*, and *T. elongatus*) exhibited more intraspecific variation than expected, suggesting potential cryptic species. Further investigation of specimens identified morphological and biogeographic differences suggesting three *Testudacarus minimus*-like species, four *T. hitchensi*-like species, and three *T. elongatus*-like species. However, some of these “species” exhibit high intraspecies COI divergence with restricted geographic ranges and no diagnosable morphological variability, and should be the target of further research.

In summary, we find strong support through a combination of morphology, biogeography, and phylogenetic analysis of COI and 28S for 17 species sorted into four robustly supported species complexes. The following species complexes are proposed to better organize the subfamily: *Testudacarus minimus* complex, *T. hitchensi* complex, *T. americanus* complex, and *T. elongatus* complex. Each complex is treated below within the taxonomic descriptions.
Figure 6. Testudacarinae molecular phylogeny and species complexes: (Left) combined 28S and COI Bayesian analysis resulting in a five branched soft polytomy (●: >95% posterior probability); monophyly tested across Torrenticolidae but not depicted; (A–E) represent tree continuation in Figs 8, 12, 23, 32, and 43 respectively; (Right) species complexes with illustrative descriptions.
Key to Testudacarinae species complexes:

1 Pedipalp four-segmented, anterior tips of coxae-I with projections ............
   ...................................................................................................................... *D. oribatoides*
– Pedipalp five-segmented, anterior tips of coxae-I without projections .... 2

2 Body elongate to rectangular................................. *T. elongatus complex*
– Body oval.........................................................................................................

3 Body large (>700 µm female and >650 male dorsal length), dull coloration common; within and west of the Rocky Mountains.........................
   ................................................................................................. *T. americanus complex (except T. rollerae)*
– Body small (<700 µm female and <650 male dorsal length), bright coloration (orange, red, violet, blue) common; present throughout North America....
   ........................................................................................................

4 Anterior-medial platelet wide (>140 µm) and more than or nearly twice as wide as long......................................................................................... *T. rollerae*
– Anterior-medial platelet unmodified (<140 µm) and far less than twice as wide as long .................................................................................................

5 Anterior-medial and anterior-lateral platelets with consistent coloration (either colored or colorless across) ........................................ *T. minimus complex*
– Anterior-lateral platelets with coloration and anterior-medial platelet colorless....
   .......................................................................................................................... *T. hitchensi complex*


**Taxonomy**

Torrenticolidae Piersig, 1902
http://zoobank.org/F4D093F6-B225-4E9B-999E-9956A9866564

*Note.* See Fisher et al. (2015) for diagnosis.

Testudacarinae Cook, 1974
http://zoobank.org/82730C11-1A78-4B39-8F74-6AD8ACF83A04

Subfamilial diagnosis. For larval diagnosis see Smith (1982). Adults differ from torrenticolines in having three pairs of acetabula (six in Torrenticolineae); condyles present over the insertions of leg-IV; long posterio-dorsal subcapitular apodemes (also long in Monatractides); a ridge extending anteriorly from the leg-IV socket; and a ring of platelets closely affiliated with the central dorsal plate, i.e., not hidden within a dorsal furrow as in torrenticolines (Fig. 1). They are further characterized by having a single antero-medial dorsal platelet and pedipalps without ventral projections, although some torrenticolines also have these characters. Testudacarineae can be further diagnosed by the following combination of characters. Medial dorsal plate exhibiting secondary and occasionally tertiary sclerotization. Dorsal platelets variable in size, shape, and coloration. Antero-medial platelet smaller than antero-lateral platelets and trapeziform (rounded to rectangular). Antero-lateral platelets long with anterior bulge and posterior tapering. Seven pairs of lateral platelets present. Lateral-platelet-2, -4, and -6 large and elongate and -1, -3, -5, and -7 smaller and rounded. Lateral-platelet-3 highly variable and positioned either anterior or lateral to lateral-platelet-4. Lateral-platelet-4 highly variable in shape mostly depending on lateral-platelet-3 position. Dorso-glandularia-2 and post-ocularial setae located together on antero-lateral platelet. Dorso-glandularia-3, -5, and -6 located on lateral-platelet-1, -5, and -7 respectively. Dorso-glandularia-4 located on the lateral platelet. Latero-glandularia-4 located at tip of coxae-I (as in Monatractides and many Torrenticola). Pedipalp, femur, and genu with plumose setae ventrally. Also similar to Monatractides, posterio-dorsal subcapitular apodemes are long. Rostrum short.

Distribution. Testudacarines have been reported on many occasions outside of their original descriptions. Furthermore, the Canadian National Collection in Ottawa, Canada includes thousands of testudacarines collected from across most of North America (Smith et al. 2010). In Asia there have only been a handful of additional reports (Walter 1929, Pešić and Smit 2007, Jin et al. 2010, Morimoto 2012). This is not completely due to a lack of torrenticolid work in Asia, for an extensive list see Walter et al. (2009, pg. 256) and Fisher et al. (2015). Extensive work has also been done on water mites in Europe, Africa, and Australia without any reports of testudacarines. Therefore, testudacarines are currently thought to be widely distributed throughout most of North America (with southern limits in Mexico and northern limits around the 60th parallel), and sparsely distributed in parts of Asia.

Remarks. The three pairs of acetabula, coxae-IV condyles, and “generalized” pedipalps are plesiomorphic states that clearly show testudacarines as retaining ancestral torrenticolid characteristics (Wiles 1997a). Wiles (1997a) and other authors suggest latero-glandularia-3 is present on the dorsum of testudacarines. However, we suggest that this is latero-glandularia-4 due to its posterior-most positioning. We also detail sexually dimorphic characters (Fig. 7). Although Habeeb (1954) first noted differences between the sexes of T. vulgaris, he did not present these distinctions in their wider context as overall conditions of Testudacarineae. Sexual dimorphism present in Testudacarineae include: 1) female dorso-glandularia-4 positioned closer to the muscle...
Figure 7. Testudacarine sexual dimorphism: female dorsal shield (A) and ventral shield (C) differing from male (B, D) by the following characters: 1) dorso-glandularia-4 positioned far closer to muscle scars; 2) area of secondary sclerotization always present (males rarely present; very small if present); 3) with shorter coxae-II+III midline; 4) genital field enveloped by coxal field; 5) larger and rounder body (males around 80% of female size); 6) excretory pore well separated from ventral line of secondary sclerotization.
scars; 2) dorsal secondary sclerotization always present in females and usually absent in males (very small if present in males); 3) female coxae-II+III midline short; 4) genital field almost entirely enveloped by coxal field in females but only around half of male genital field within coxal field; 5) females idiosoma larger and rounder (males around 80% of female size) with less of the ventral shield composed of coxal field; and 6) excretory pore well separated from ventral line of secondary sclerotization in females, and is either in direct contact with or nearly so in males.

**Debsacarus** Habeeb, 1974  
http://zoobank.org/9C344329-32F6-4C4B-8167-196E030B2ED8


**Type species.** *Debsacarus oribatoides* (Habeeb, 1961).

**Generic diagnosis.** *Debsacarus* differ from all other Testudacarinae in having four-segmented pedipalps (instead of five) and projections on the anterio-tips of coxae-I. With the exception of *Testudacarus hyporhynchus*, *Debsacarus* differ from all other Testudacarinae in having an elongate gnathosoma and an extremely narrow gnathosomal bay that is covered dorsally and ends anterior to the leg-I insertion ventrally.

**Distribution.** Known from only two counties (Los Angeles and Monterey) in California.

**Debsacarus oribatoides** (Habeeb, 1961)  
http://zoobank.org/7749B09F-CA26-416A-8FE7-F445A5451B85


**Type series.** Lectotype (1♀): California, USA: 1♀ from Los Angeles County, Coldbrook Guard Station, North Fork of San Gabriel River, 25 June 1961, by H Habeeb, HH610024; Paralectotype (1♂): California, USA: 1♂ from Los Angeles County, Coldbrook Guard Station, North Fork of San Gabriel River, 25 June 1961, by H Habeeb, HH610024.

**Other material examined.** Other (10♀, 8♂): California, USA: 1♂ from Monterey County, Salmon Falls Creek, beside Route 1 12.5 km south of Gorda (35°48’56.00”N, 121°21’30.00”W), 2 June 2010, by IM Smith, IMS100045; 5♀ and 3♂ from Monterey County, Los Padres National Forest, Lucia beside Ferguson-Nacimiento Road 5.6 km east of Route 1 (36°0’3.00”N, 121°28’31.00”W), 3 June 2010, by IM Smith, IMS100048; 1♀ and 3♂ from Monterey County, Los Padres National Forest, Lucia beside Nacimiento-Ferguson Road 11.3 km west of Nacimiento Campground (36°1’N,
Figure 8. Debsacarus oribatoides molecular phylogeny: 28S and COI Bayesian analysis showing strong support single distinct clade (●: >95% posterior probability); clade exhibits <.6% divergence in COI within and >15% divergence between any other clade (not pictured); continuation of (E) lineage from Fig. 6.

121°27′W), 30 July 1987, by IM Smith, IMS8700119; 1♀ and 1♂ from Monterey County, Los Padres National Forest, Salmon Creek, beside Route 1 south of Gorda (35°49′N, 121°22′W), 29 July 1987, by IM Smith, IMS870118; 1♀ from Monterey County, Limekiln State Park, Hare Canyon Creek, near campground (36°0′41.00″N, 121°31′1.00″W), 6 September 2013, by JR Fisher, JRF13-0906-001; 1♀ from Monterey County, Salmon Creek, beside Route 1 south of Gorda (35°49′N, 121°22′W), 28 July 1987, by IM Smith, IMS870114A; 1♀ from Los Angeles County, Angeles National Forest, North Fork of San Gabriel River, off Route 39 (34°16′16.00″N, 117°50′46.00″W), 8 September 2013, by JR Fisher, JRF13-0908-001.

Type deposition. Lectotype (1♀), and paralectotype (1♂) deposited at CNC.

Redescription. Female (n=11) with characteristics of the genus with following specifications.


Venter (Fig. 10) — [779–929 long; 510–610 wide] round to ovoid and colorless. Primary sclerotization [668–756 long]. Gnathosomal bay [33–45 dorsal length; 128–148 ventral length; 33–38 wide] very narrow; dorsal bay length extremely short giving the bay


Male (n=9) similar to female except for sexually dimorphic characters previously discussed and with following specifications.


**Diagnosis.** Same as genus.
**Distribution.** Same as genus.

**Remarks.** *Debsacarus oribatoides* show at least 15% COI divergence from all other Testudacarinae and less than .6% divergence from one another (Fig. 8). Additionally, Habeeb (1961) describes a protrusible maxillary tube, however, we find no evidence in the additional specimens examined that the maxillary tube or subcapitulum is any more protrusible than what is commonly found in other *Testudacarus*, and certainly is not protrusible like in *Pseudotorrenticola*. Habeeb (1961) did not designate types, however, he described the species from the only two specimens available. From those two specimens, we have designated a lectotype (♀) and paralectotype (♂).

*Testudacarus* Walter, 1928

http://zoobank.org/F535321F-2CB2-4F9D-B955-659A39CC564D

Type species. *Testudacarus tripeltatus* Walter, 1928

**Generic diagnosis.** Members of this genus, unlike *Debsacarus*, lack projections on the anterior tips of coxae-I and have five-segmented pedipalps (instead of four). Furthermore, with the exception of *Testudacarus hyporhynchus*, they differ from *Debsacarus* in having a rounded gnathosoma (rather than elongate) and a wide gnathosomal bay that is uncovered dorsally and ventrally ends posterior to the leg-I insertion.

**Distribution.** Same as subfamily.

*Testudacarus minimus* complex

**Species complex diagnosis.** These species can be distinguished from most other testudacarines by their small size (female and male dorsal length less than 700 and 600 µm, respectively), highly variable coloration (red, orange, blue, violet, and rarely colorless), and small (<140 µm), rounded anterio-medial platelet (differing from *Testudacarus rollerae*, which has a large (>140 µm) anterio-medial platelet more than or nearly twice as wide as long). Additionally, only this complex and the *T. hitchensi* complex are pre-
Figure 12. Testudacarus minimus complex molecular phylogeny: 28S and COI Bayesian analysis showing strong support for a soft polytomy with three distinct clades (●: >95% posterior probability); colored clades exhibit <2.5% divergence in COI within and >6.5% divergence between; continuation of (A) lineage from Fig. 6.
sent east of the Great Plains. These two complexes resemble each other morphologically in many respects, but can be easily distinguished because members of this complex have uniform coloration across all three anterior platelets while *T. hitchensi*-like mites have a colorless antero-medial platelet and colored antero-lateral platelets. With the exception of *T. radwellae*, males of this complex differ from *T. hitchensi*-like mites in having dorso-glandularia-4 positioned less anterior to and more lateral to the muscle scars. This complex is abundant and present across most of North America and comprises the following species: *T. deceptivus*, *T. minimus*, *T. radwellae*, and *T. vulgaris*.

**Remarks.** Molecular data show strong support for three distinct clades (Fig. 12). All three clades exhibit less than 2.5% COI divergence within the clade and greater than 6.5% divergence between clades. In California there is currently no reliable way to diagnose these three clades morphologically as they are all roughly the same size and color (colorless to orange). However, outside of California it is possible to diagnose clades based on color, size, and geographic distribution. Members of this complex exhibit the broadest geographic ranges and thus exhibit the highest and not unexpected intraspecies divergence of the four complexes. Given the broad geographic sampling conducted in this complex, we feel comfortable designating the three main clades, exhibiting intra-clade divergence of more than 6.5%, as multiple species: *T. minimus*, *T. vulgaris*, and *T. deceptivus*. A fourth species, *T. radwellae*, belongs to this complex based on morphology, but genetic extractions were unsuccessful. *Testudacarus radwellae* males also share morphological similarities with *T. hitchensi*-like mites (the positioning of dorso-glandularia-4). Therefore *T. radwellae* is potentially important in discovering the relationship between these two species complexes and deserves further investigation.

*Testudacarus minimus* Marshall, 1943
http://zoobank.org/CD1D1B50-6A37-4099-86D7-A6DAB8A17CA6


**Type series.** Holotype (1♂): California, USA: 1♂ from Santa Cruz County, Waddell Creek, 30–31 August 1933, by PR Needham, RM330016.

**Other material examined.** Other (15♀, 15♂): Montana, USA: 2♂ from Ravalli County, Bitterroot National Forest, Lost Horse River, downstream of confluence of North Lost Horse (45°7'7.00"N, 114°18'0.00"W), 3 August 2012, by JR Fisher and WA Nelson, ROW12-0803-006; 1♂ from Powell County, Monture Creek, at
fishing access off Highway 200 west of Ovando (47°2′15.00″N, 112°13′12.00″W), 9 August 2012, by AJ Radwell and JA Hinsey, AJR12-0809-415A; **Washington, USA:** 2♂ from Snohomish County, Mount Baker National Forest, Clean Creek, (48°13′8.00″N, 121°34′7.00″W), 28 July 2013, by JC O’Neill and WA Nelson, JNOW13-0728-007; 2♀ from Jefferson County, Olympic National Forest, Snow Creek, (47°56′11.00″N, 122°56′53.00″W), 22 July 2013, by WA Nelson and JC O’Neill, JNOW13-0722-001; 2♀ from Grays Harbor County, Capitol State Forest, Porter Creek, (46°58′13.00″N, 123°16′2.00″W), 25 July 2013, JC O’Neill and WA Nelson, JNOW13-0725-005; 1♀ from Skamania County, Gifford Pinchot National Forest, Lewis Creek, (46°7′40.00″N, 121°59′24.00″W), 1 August 2013, by JC O’Neill and WA Nelson, JNOW13-0801-004; **California, USA:** 1♂ from Inyo County, Inyo National Forest, Bishop Creek, downstream of campground (37°17′23.00″N, 118°33′14.00″W), 2 September 2013, by JR Fisher, JRF13-0902-003; 2♀ from Nevada County, Tahoe National Forest, Sagehen Creek, off Route 89 (39°26′2.00″N, 120°12′17.00″W), 26 August 2013, by JR Fisher, JRF13-0826-006; 1♀ from Siskiyou County, Klamath National Forest, Shadow Creek, off Cecilville Road, (41°12′13.00″N, 123°4′18.00″W), 17 August 2013, by JR Fisher, JRF13-0817-002; **Wyoming, USA:** 1♂ from Albany County, North Fork of Little Laramie River, at bridge on Highway 130 (41°19′42.00″N, 106°9′42.00″W), 3 August 2012, by AJ Radwell and JA Hinsey, AJR12-0803-406; 2♂ from Albany County, South Clear Creek, across from Southfork Campground on Highway 16 (44°16′36.00″N, 106°57′4.00″W), 14 August 2012, by AJ Radwell and JA Hinsey, AJR12-0814-419; 1♀ from Fremont County, Wind River, off County Road 773 30 miles east of Moran on Highway 26/287 (43°43′5.00″N, 110°48′0.00″W), 5 August 2012, by AJ Radwell and JA Hinsey, AJR12-0805-410; **Utah, USA:** 2♂ from Cache County, Wasatch-Cache National Forest, Jordan River, (41°44′33.00″N, 111°45′57.00″W), 24 July 2012, by JR Fisher and WA Nelson, ROW12-0724-004; **Idaho, USA:** 2♂ from Blaine County, Sawtooth National Forest, Baker Creek, (43°45′28.00″N, 114°33′44.00″W), 27 July 2012, by JR Fisher and WA Nelson, ROW12-0728-001; 2♂ from Lemhi County, Salmon National Forest, Niapas Creek at confluence with Panther Creek, (45°8′15.00″N, 114°13′4.00″W), 2 August 2012, by JR Fisher and WA Nelson, ROW12-0802-003; **Colorado, USA:** 1♀ from Gunnison County, Quartz Creek, north of Ohio City on County Road 76 mile marker 11 (38°34′2.00″N, 106°34′6.00″W), 1 August 2012, by AJ Radwell and JA Hinsey, AJR12-0801-403A; **Oregon, USA:** 1♀ from Tillamook County, Siuslaw National Forest, Alder Creek, (45°9′27.00″N, 123°47′60.00″W), 6 August 2013, by JC O’Neill, JNOW13-0806-002; 1♀ from Lane County, Gate Creek, (44°8′48.00″N, 122°34′20.00″W), 11 August 2013, by JC O’Neill and WA Nelson, JNOW 13-0811-001; 1♀ from Curry County, Rogue River National Forest, Elk River, off National Forest Road 5325 (42°42′46.00″N, 124°18′41.00″W), 13 August 2013, by JR Fisher, JRF13-0813-003; **Arizona, USA:** 1♀ from Cochise County, Chiricahua Mountains west of Portal, East Turkey Creek, off Forest Road 42 above junction with Forest Road 42B (31°54′32.00″N, 109°15′11.00″W), 15 May 2011, by IM Smith, IMS110003; 1♀ from Cochise County, Chiricahua Mountains west of Portal, East Turkey Creek,
off Forest Road 42 just above junction with Forest Road 42B (31°54′32.00″N, 109°15′11.00″W), 15 May 2011, by IM Smith, IMS110004.

**Type deposition.** Holotype (1♂) deposited at the CNC.

**Diagnosis.** *Testudacarus minimus* most resemble *T. vulgaris* and *T. deceptivus*. Throughout the majority of their shared range in the west, *T. minimus* are orange to red and *T. vulgaris* are violet to blue. While these two species have overlapping size ranges, *T. minimus* are generally larger. *Testudacarus vulgaris* females rarely exhibit a dorsal length over 600 µm and males rarely exceed 500 µm while *T. minimus* females and males are usually larger than 600 and 500 µm, respectively. *Testudacarus deceptivus* have only been found in two counties in California and cannot be distinguished from either *T. minimus* or *T. vulgaris* using morphology. *Testudacarus minimus* are the only members of their complex that have been found in Washington and northern Oregon.

**Redescription. Female (n=14)** with characteristics of the genus with following specifications.


Legs — colorless, or with same color as dorsal plate. Total leg and podomere lengths as follows: Leg-I [428–477 total; trochanter 48–55; basifemur 72–85; telofemur 60–69; genu 78–90; tibia 83–95; tarsus 79–92]. Leg-II [453–530 total; trochanter 54–62;

**Male (n=16)** similar to female except for sexually dimorphic characters previously discussed and with following specifications.


Figure 14. *Testudacarus minimus* color variation.


Legs — total leg and podomere lengths as follows: Leg-I [435–483 total; trochanter 53–63; basifemur 75–84; telofemur 57–69; genu 78–89; tibia 82–93; tarsus 80–90]. Leg-II [458–518 total; trochanter 52–64; basifemur 75–87; telofemur 59–69; genu 79–90; tibia 92–104; tarsus 96–109]. Leg-III [530–599 total; trochanter 54–62; basifemur 75–88; telofemur 63–72; genu 97–111; tibia 114–133; tarsus 124–137]. Leg-IV [722–813 total; trochanter 81–95; basifemur 102–122; telofemur 103–118; genu 130–159; tibia 150–167; tarsus 145–158].
Systematics of testudacarine torrent mites (Acari, Hydrachnidia, Torrenticola...)

Distribution. Abundant throughout North America, ranging from the Pacific Northwest to the southwestern United States (and potentially into northern Mexico), and east into the western Great Plains.

Remarks. Commonly colorless or orange in the southwestern United States; red, pink, or orange–red in the northwest, Rocky Mountains, and western Great Plains; and uncommonly red–violet in the northwest, Rocky Mountains, and western Great Plains.

Testudacarus vulgaris Habeeb, 1954
http://zoobank.org/AD09023D-849F-4F13-BD0C-1CF7B6623748


Type series. Syntypes (1♀, 1♂): New Brunswick, Canada: from Victoria County, Salmon River, 21 June 1953, by H. Habeeb, 87-53

Other material examined. Other (18♀, 19♂): Ontario, Canada: 1♀ and 1♂ from Lennox and Addington County, Hydes Creek, beside Highway 41 23.7km north of Highway 28 at Denbigh (45°11’22.00"N, 77°13’38.00"W), 29 April 2010, by IM Smith, IMS100023; 1♂ from Hastings County, Maple Leaf and Papineau Creek, east of Davis Road before Highway 62, 18 August 2011, by IM Smith, IMS110053; New Brunswick, Canada: 2♀ and 1♂ from Victoria County, Little Wapske River, Plaster Rock beside Highway108 20.5km east of Highway109, 5 September 2011, by IM Smith, IMS110061; Nova Scotia, Canada: 1♂ from Inverness County, Cheticamp River, 10 September 2011, by IM Smith, IMS110071; Tennessee, USA: 1♀ and 1♂ from Monroe County, Turkey Creek, beside Forest Road #210 just east of Forest Road #35 7.1km southeast of Route 165 (35°20’28.00"N, 84°11’30.00"W), 12 September 2009, by IM Smith, IMS090110; 2♂ from Sevier County, Great Smoky Mountains Nation Park, Rhododendron Creek, beside Greenbrier Road 2.2 km south of Route 321 (35°43’32.00"N, 83°24’2.00"W), 2 September 2009, by IM Smith, IMS090093; North Carolina, USA: 2♀ and 1♂ from Haywood County, Great Smoky Mountains National Park, Big Creek, Waterville Big Creek Picnic Area (35°44’59.00"N, 83°6’42.00"W), 16 September 2010, by IM Smith, IMS100138; 1♀ and 1♂ from Haywood County, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road near bridge 1.7km north of road to campground (35°38’45.00"N, 83°4’32.00"W), 20 September 2010, by IM Smith, IMS100150; South Dakota, USA: 1♀ and 1♂ from Lawrence County, Jim Creek, south of Nemo Road on Goodhope Road behind cab at Green Mountain Black Hills (44°9’9.00"N, 103°28’51.00"W), 15 August 2012, by AJ Radwell and JA Hinsey, AJR12-0815-421; Colorado, USA: 1♂ from San Miguel County, San Miguel River, beside Route 145 12.5km northwest of junction with road to Telluride (37°59’17.00"N, 107°59’34.00"W), 31 July 2012, by AJ Radwell and JA Hinsey, AJR12-0731-400; Pennsylvania, USA: 1♂ from Fayette County, Ohiophyle State Park, Laurel Run, fishing access #2 off T798 (Meadow Run Road) (39°50’58.00"N, 79°30’51.00"W), 10 August 2014, by MJ Skvarla, MS14-0810-005; 2♀ and 2♂ from Fayette County, State Game Lands #51, Dunbar Creek, off Furnace Hill Road East of Dunbar (39°56’16.10"N, 79°35’3.70"W), 10 August 2014, by MJ Skvarla, MS14-0810-002; California, USA: 1♂ from Monterey County, Andrew Molera State Park, Big Sur River, off Route 1 (36°16’31.00"N, 121°49’14.00"W), 4 September 2013, by JR Fisher, JRF13-0904-003; 1♂ from Inyo County, Inyo National Forest, Bishop Creek, downstream of campground (37°17’23.00"N, 118°33’14.00"W), 2 September 2013, by JR Fisher, JRF13-0902-003; 1♂ from Alpine County, Markleeville Creek, off Route 89 downstream of bridge (38°41’39.00"N, 119°46’41.00"W), 30 August 2013, by JR Fisher, JRF13-0830-001; 1♂ from Mendocino County, Jackson Dem-
onstration State Park, North Fork of Big River, (39°20′46.00″N, 123°30′35.00″W), 22 August 2013, by JR Fisher, JRF13-0822-002; 1♀ from Mono County, Humboldt-Toiyabe National Forest, Little Walker River, off Route 108 downstream of tunnel (38°20′57.00″N, 119°27′15.00″W), 31 August 2013, by JR Fisher, JRF13-0831-002; 1♀ from Trinity County, Shasta-Trinity National Forest, North Fork of Trinity River, (40°46′47.00″N, 123°7′46.00″W), 18 August 2013, JRF13-0818-005; **Oregon, USA:** 2♂ from Douglas County, Umpqua National Forest, Calf Creek, (43°17′28.00″N, 122°37′12.00″W), 12 August 2013, by JC O’Neill and WA Nelson, JNOW13-0812-006; **Utah, USA:** 2♀ from Utah County, Uinta National Forest, Hobble Creek, just upstream on right fork Hobble Creek Road from Cherry Campground (40°10′9.00″N, 111°28′26.00″W), 22 July 2012, by JR Fisher and WA Nelson, ROW12-0722-001; **Idaho, USA:** 1♀ from Fremont County, Targhee National Forest, Rock Creek, downstream of tributary (44°6′44.00″N, 111°15′4.00″W), 25 July 2012, by JR Fisher and WA Nelson, ROW12-0725-001; **Arkansas, USA:** 1♀ from Searcy County, Tomahawk Creek, (36°1′20.00″N, 92°40′43.00″W), 20 July 2009, by AJ Radwell, AJR090101.

**Type deposition.** Syntypes (1♀, 1♂) deposited at the CNC.

**Diagnosis.** *Testudacarus vulgaris* most resemble *T. minimus* and *T. deceptivus*. Throughout the majority of their shared range in the west, *T. minimus* are orange to red and *T. vulgaris* are violet to blue. While these two species have overlapping size ranges, *T. minimus* are generally larger. *Testudacarus vulgaris* females rarely exhibit a dorsal length over 600 µm and males rarely exceed 500 µm while *T. minimus* females and males are usually larger than 600 and 500 µm, respectively. *Testudacarus deceptivus* have only been found in two counties in California and cannot be distinguished from either *T. minimus* or *T. vulgaris* using morphology. *Testudacarus vulgaris* are the only members of their complex that have been found east of the Great Plains.

**Redescription. Female (n=18)** with characteristics of the genus with following specifications.


Legs — colorless, or with same color as dorsal plate. Total leg and podomere lengths as follows: Leg-I [401–497 total; trochanter 50–61; basifemur 74–85; telofemur 55–72; genu 72–96; tibia 75–97; tarsus 78–97]. Leg-II [417–564 total; trochanter 51–63; basifemur 71–92; telofemur 57–72; genu 75–100; tibia 92–118; tarsus 96–120]. Leg-III [513–664 total; trochanter 55–68; basifemur 71–96; telofemur 58–82; genu 91–124;
Figure 17. *Testudacarus vulgaris* color variation.


**Male (n=17)** similar to female except for sexually dimorphic characters previously discussed and with following specifications.


**Distribution.** Abundant throughout the majority of North America. Unreported in Washington and northern Oregon.
Remarks. Commonly orange and uncommonly violet in the southwestern United States; commonly violet or blue and uncommonly red–violet in the Rocky Mountains and Great Plains; commonly violet or blue east of the Great Plains.

Testudacarus deceptivus O’Neill & Dowling, sp. n.
http://zoobank.org/13FDE612-2F95-4498-939E-E95CAD6403CD

Type series. Holotype (1♀): California, USA: 1♀ from Los Angeles County, Angeles National Forest, North Fork of San Gabriel River, off Route 39 (34°16’16.00”N, 117°50’46.00”W), 8 September 2013, by JR Fisher, JRF13-0908-001 (Specimen 143652 – DNA#2078); Paratype (1♂): California, USA: (allotype) 1♂ from Sierra County, Tahoe National Forest, Milton Creek near confluence of North Yuba River, (39°34’4.00”N, 120°36’54.00”W), 25 August 2013, by JR Fisher, JRF13-0825-004 (Specimen 143666 – DNA#2091)

Type deposition. Holotype (1♀) and allotype (1♂) deposited at the CNC.

Diagnosis. Testudacarus deceptivus have only been found in two counties (Los Angeles and Sierra) in California and cannot be distinguished from either T. minimus or T. vulgaris using morphology.

Description. Female (n=1) with characteristics of the genus with following specifications.


Dorsum (Fig. 19) — [597 long; 468 wide] ovoid and colorless. Dorsal plate [500 long; 410 wide]. Primary sclerotization [420 long]. Dorso-glandularia-4 [244 apart] in line with and well lateral to [78] muscle scars. Platelets completely colorless. Anterio-medial platlet [133 long; 74 wide]. Anterio-lateral platelet [168 long; 70 wide]. Lateral platelets as follows: lateral-1 [54 long; 43 wide]; lateral-2 [126 long; 31 wide]; lateral-3 [42 long; 20 wide]; lateral-4 [115 long; 29 wide]; lateral-5 [45 long; 27 wide]; lateral-6 [89 long; 30 wide]; lateral-7 [62 long; 27 wide].

Venter (Fig. 19) — [777; 521 wide] ovoid and colorless. Primary sclerotization [600 long]. Gnathosomal bay [76 dorsal length; 145 ventral length; 60 wide]. Coxal field [458 long; 336 wide]. Coxa-I [248 long; 102 midlength]. Coxa-II + III [117 distance to top of coxa-II; 192 distance to top of coxa-III; 340 distance to bottom of coxa-III; 223 total length]. Coxa-IV [322 distance to top; 136 total length]. Genital field [318 distance to top; 479 distance to bottom; 160 total length; 133 width; 173 distance from gnathosomal bay; 70 distance from coxa-I; 188 distance to excretory pore; 299 distance to caudad]. Distance to excretory pore [666].

Legs — colorless. Total leg and podomere lengths as follows: Leg-I [480 total; trochanter 62; basifemur 80; telofemur 64; genu 91; tibia 92; tarsus 90]. Leg-II [519...
Figure 19. *Testudacarus deceptivus* female: (Left) dorsum; (Right) venter. Scale: 100 µm.

Male (n=1) similar to female except for sexually dimorphic characters previously discussed and with following specifications.

Gnathosoma — Subcapitulum [139 ventral length; 90 dorsal length; 83 tall]. Chelicerae [125 long]. Fangs [29 long]. Pedipalp [179 long]. Trochanter [26 long; 29 wide]. Femur [48 long; 35 wide]. Genu [40 long; width 29 wide]. Tibia [44 long; 23 wide]. Tarsus [20 long; 10 wide].

Dorsum (Fig. 20) — [470 long; 350 wide]. Dorsal plate [397 long; 317 wide]. Dorso-glandularia-4 [169 apart] anterior [39] and lateral to [47] muscle scars. Antero-medial platelet [105 long; 67 wide]. Antero-lateral platelets [154 long; 62 wide]. Lateral platelets as follows: lateral-1 [36 long; 29 wide]; lateral-2 [90 long; 20 wide]; lateral-3 [36 long; 14 wide]; lateral-4 [70 long; 20 wide]; lateral-5 [39 long; 15 wide]; lateral-6 [59 long; 16 wide]; lateral-7 [44 long; 16 wide].

total; trochanter 63; basifemur 83; telofemur 69; genu 94; tibia 104; tarsus 106]. Leg-III [615 total; trochanter 63; basifemur 85; telofemur 72; genu 115; tibia 133; tarsus 145]. Leg-IV [821 total; trochanter 93; basifemur 112; telofemur 122; genu 161; tibia 178; tarsus 155].
Figure 20. *Testudacarus deceptivus* male: (Left) dorsum; (Right) venter. Scale: 100 µm.

Venter (Fig. 20) — [600; 386 wide]. Primary sclerotization [554 long]. Gnathosomal bay [54 dorsal length; 131 ventral length; 52 wide]. Coxa field [413 long; 290 wide]. Coxa-I [219 long; 88 midlength]. Coxa-II + III [96 distance to top of coxa-II; 168 distance to top of coxa-III; 331 distance to bottom of coxa-III; 235 total length]. Coxa-IV [291 length to top; 122 total length]. Genital field [354 distance to top; 491 distance to bottom; 137 total length; 107 width; 223 distance from gnathosomal bay; 135 distance from coxa-I; 63 distance to excretory pore; 91 distance to caudad]. Genital skeleton [192 long; 89 wide]. Distance to excretory pore [554].

Legs — total leg and podomere lengths as follows: Leg-I [413 total; trochanter 51; basifemur 69; telofemur 61; genu 73; tibia 79; tarsus 78]. Leg-II [462 total; trochanter 60; basifemur 75; telofemur 59; genu 80; tibia 94; tarsus 93]. Leg-III [517 total; trochanter 56; basifemur 73; telofemur 65; genu 95; tibia 111; tarsus 116]. Leg-IV [688 total; trochanter 76; basifemur 97; telofemur 97; genu 132; tibia 146; tarsus 138].

**Etymology.** Specific epithet *deceptivus* (*decept-*, L. deceptive) refers to the lack of morphological characters differentiating this species from related species.

**Distribution.** Known from only two counties (Los Angeles and Sierra) in California.
Testudacarus radwellae O’Neill & Dowling, sp. n.
http://zoobank.org/D9D64AA5-FBE6-4156-BB22-FC4A0B834D96

Type series. Holotype (1 ♀): Arkansas, USA: 1 ♀ from Montgomery County, Ouachita National Forest, Collier Springs, at spring structure picnic area (34°29′7.04″N, 93°35′38.12″W), 11 November 2009, by AJ Radwell, AJR090317C (Specimen 144016); Paratypes (1 ♀, 7 ♂): Arkansas, USA: (allotype) 1 ♂ from Montgomery County, Ouachita National Forest, Collier Springs, at spring structure picnic area (34°29′7.04″N, 93°35′38.12″W), 29 July 2011, by AJ Radwell and B Crump, AJR110301 (Specimen 144011); 4 ♂ from Montgomery County, Ouachita National Forest, Collier Springs, at spring structure picnic area (34°29′7.04″N, 93°35′38.12″W), 29 July 2011, by AJ Radwell and B Crump, AJR110301; 1 ♂ from Polk County, Ouachita National Forest, upper small pond on stream running along trail (34°27′36.73″N, 93°59′52.38″W), 21 July 2008, by AJ Radwell, AJR080303A; 1 ♂ from Montgomery County, Ouachita National Forest, Collier Springs, picnic area beside Forest Road 177 (34°29′3.00″N, 93°35′35.00″W), 19 September 2008, by IM Smith, IMS080061A; 1 ♀ from Montgomery County, Ouachita National Forest, Collier Springs, at spring structure picnic area (34°29′7.04″N, 93°35′38.12″W), 11 November 2009, by AJ Radwell, AJR090317C.

Type deposition. Holotype (1 ♀), allotype (1 ♂), and three paratypes (3 ♂) deposited at the CNC; four paratypes (1 ♀, 3 ♂) at the ACUA.

Diagnosis. Testudacarus radwellae and T. vulgaris are the only testudacarines known to occur in Arkansas. Testudacarus radwellae are conspicuously violet over the entirety of their body, whereas the violet coloration of T. vulgaris is less vibrant and often absent, particularly on the platelets, legs, and secondary sclerotization of the venter. Males of T. radwellae also have dorsal-glandularia-4 located far lateral to the muscle scars, unlike others in the complex.

Description. Female (n=2) with characteristics of the genus with following specifications.


Male (n=7) similar to female except for sexually dimorphic characters previously discussed and with following specifications.


Venter (Fig. 22) — [575–606 long; 369–400 wide]. Primary sclerotization [536–555 long]. Gnathosomal bay [49–66 dorsal length; 130–137 ventral length; 48–56 wide]. Coxal field [405–424 long; 281–305 wide]. Coxa-I [223–238 long; 90–102 midlength]. Coxa-II + III [100–113 distance to top of coxa-II; 156–169 distance to
top of coxa-III; 326–346 distance to bottom of coxa-III; 223–244 total length]. Coxa-IV [270–283 length to top; 126–146 total length]. Genital field [343–366 distance to top; 485–510 distance to bottom; 139–146 total length; 115–123 width; 210–232 distance from gnathosomal bay; 90–102 distance from coxa-I; 44–54 distance to excretory pore; 87–101 distance to caudad]. Genital skeleton [179–182 long; 94–103 wide]. Distance to excretory pore [536–555].


**Etymology.** Specific epithet *radwellae* after the late Dr Andrea J. Radwell, the American water mite researcher who collected the specimens needed for this description. Dr Radwell collaborated with us on the larger torrenticolid project as a whole, giving us invaluable advice and mentorship. Without her, large portions of this project would not have been possible. She is dearly missed.

**Distribution.** Reported from only two counties (Polk and Montgomery) in Arkansas.

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**Testudacarus hitchensi complex**

**Species complex diagnosis.** Only this complex and the *T. minimus* complex are present east of the Great Plains. These two complexes resemble each other morphologically in many respects, but can be easily distinguished because members of this complex have non-uniform coloration across all three anterior platelets (colorless antero-medial platelet and colored anterio-lateral platelets) while *T. minimus*-like mites possess uniform coloration across all three platelets. Males of this complex differ from *T. minimus*-like mites in having dorso-glandularia-4 positioned more anterior to and less lateral to the muscle scars. These mites are common in eastern United States and rare in eastern Canada and Florida, small (female and male dorsal length less than 700 and 600 µm, respectively), and violet to blue in color. This complex comprises the following species: *T. harrisi*, *T. dennetti*, *T. dawkinsi*, and *T. hitchensi*.

**Remarks.** Distinguishable morphological characters can be found for four lineages while genetic data indicates more diversity (Fig. 23), suggesting cryptic speciation within the clade. Three clades (violet and blue clades in Fig. 23) exhibit less than 1.5% COI divergence within the clade and greater than 6% divergence between clades. This relatively low divergence within clades over their large ranges compared to the high divergence exhibited between clades even in the same streams strongly supports multiple species. The fourth clade (green in Fig. 23) proves problematic as no morphological variability has been found within the clade, but COI divergence of up to 4.5% is present and within a small geographic area (North Carolina and Tennessee). Ethanol
Figure 23. *Testudacarus hitchensi* complex molecular phylogeny: 28S and COI Bayesian analysis showing strong support for at least four distinct clades, but suggesting more (●: >95% posterior probability); excepting green clade, clades exhibit <1.5% divergence in COI within and >6% between; green clade exhibits <4.5% within and >9.5% between other clades; specimens in red constitute additional suspected species based on genetic data, but lack morphological or distributional variation from green clade; continuation of (B) lineage from Fig. 6.
collections were limited from this region and more data is needed. Furthermore, examinations of GAW collections provided by the CNC suggest there are other potential “morphotypes” of this species complex unrepresented in the genetic data presented. More species almost certainly exist in this complex, and further research is needed.

*Testudacarus hitchensi* O’Neill & Dowling, sp. n.
http://zoobank.org/0A6954E1-84CF-4F79-B966-B9F6E0587739

**Type series. Holotype (1♀): North Carolina, USA:** 1♀ from Haywood, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road at Hannah Hoglen Cemetery site (35°38′29.00″N, 83°3′22.00″W), 22 September 2010, by IM Smith, IMS100154 (Specimen 141898 – DNA#1493); **Paratypes (9♀, 10♂): North Carolina, USA:** (allotype) 1♂ from Haywood, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road at Hannah Hoglen Cemetery site (35°38′29.00″N, 83°3′22.00″W), 22 September 2010, by IM Smith, IMS100154 (Specimen 146756 – DNA#2171); 1♀ and 2♂ from Haywood, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road at Hannah Hoglen Cemetery site (35°38′29.00″N, 83°3′22.00″W), 22 September 2010, by IM Smith, IMS100154; 2♀ and 1♂ from Haywood County, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road near bridge 1.7km north of road to campground (35°38′45.00″N, 83°4′32.00″W), 20 September 2010, by IM Smith, IMS100150; 2♂ from Macon County, Rainbow Springs, beside Forest Road 67 4.4 km south of Standing Indian Campground (35°3′6.00″N, 83°30′45.00″W), 1 July 2006, by IM Smith, IMS060040; 2♂ from Yancey County, Pisgah National Forest, South Toe River, Lost Cove beside Toe River Road (Forest Road 472) 0.4km east of Forest Road 2074 (35°45′0.00″N, 82°12′53.00″W), 9 September 2007, IM Smith, IMS070059; 1♀ from Yancey County, Pisgah National Forest, South Toe River, Lost Cove Picnic Area beside Toe River Road (Forest Road 472) 2.8 km east of Route 80 (35°45′13.00″N, 82°12′42.00″W), 27 September 2009, by IM Smith, IMS090127; **Tennessee, USA:** 1♂ from Monroe, beside Forest Route #35 2.3km northeast of road from Route 165 to Miller Chapel Baptist Church (35°21′47.00″N, 84°9′47.00″W), 12 September 2009, by IM Smith, IMS090127; **Georgia, USA:** 1♀ from Floyd County, beside road from Everett Springs to Villanow 1.4 km south of The Pocket Recreation Area, 4 July 1990, by IM Smith, IMS900077.

Paratyp e examinations but measurements not included. (1♀, 2♂): North Carolina, USA: 1♀ from Haywood County, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road near bridge 1.7km north of road to
campground (35°38′45.00″N, 83°4′32.00″W), 20 September 2010, by IM Smith, IMS100150; 1♂ from Haywood County, Great Smoky Mountains National Park, tributary of Hemphill Creek, Appalachian Highlands Science Learning Center near Ferguson Cabin site, (35°34′56.00″N, 83°4′30.00″W), 21 September 2010, by IM Smith, IMS100153; Tennessee, USA: 1♀ from Sevier County, Great Smoky Mountains National Park, Catron Branch, Elkmont Road off Little River Road (35°39′51.00″N, 83°35′19.00″W), 24 September 2010, IMS100156.

**Type deposition.** Holotype (1♀), allotype (1♂), and eight paratypes (4♀, 4♂) deposited at CNC; ten paratypes (5♀, 5♂) at ACUA.

**Diagnosis.** These mites differ from all others in the complex in having large medial pores on the dorsal plate surrounded by a ring of smaller pores (all pores uniform in other species). Males also have a “bleached” or colorless area posterior to the coxal plate that is colored in other members of the complex.

**Description. Female (n=10)** with characteristics of genus with following specifications.


**Legs** — orange and restricted violet to blue. Total leg and podomere lengths as follows: Leg-I [473–524 total; trochanter 60–62; basifemur 83–93; telofemur 65–76;
Systematics of testudacarine torrent mites (Acari, Hydrachnidia, Torrenticolidae)...  

**Figure 24.** *Testudacarus hitchensi* female: (Left) dorsum; (Right) venter. Scale: 100 µm.


Male (n=10) similar to female except for sexually dimorphic characters previously discussed and with following specifications.


Figure 25. *Testudacarus hitchensi* male: (Left) dorsum; (Right) venter. Scale: 100 µm.

50 long; 19–32 wide; lateral-4 [74–110 long; 30–35 wide]; lateral-5 [46–58 long; 25–33 wide]; lateral-6 [53–75 long; 27–34 wide]; lateral-7 [46–62 long; 24–33 wide].


Legs — total leg and podomere lengths as follows: Leg-I [444–508 total; trochanter 55–62; basifemur 75–89; telofemur 63–73; genu 80–91; tibia 85–99; tarsus 84–96]. Leg-II [474–533 total; trochanter 60–64; basifemur 77–90; telofemur 61–71; genu 82–93; tibia 92–106; tarsus 99–113]. Leg-III [537–598 total; trochanter 57–64; basifemur 80–92; telofemur 65–73; genu 96–108; tibia 113–128; tarsus 121–137].
Leg-IV [721–778 total; trochanter 88–99; basifemur 96–117; telofemur 102–113; genu 135–151; tibia 147–168; tarsus 142–156].

**Etymology.** Specific epithet *hitchensi* after the late Christopher Eric Hitchens, the English author, journalist, and literary critic. As Sam Harris’ wife, Annaka, said: “Nothing Hitchens does is ever boring.” Hitchens has inspired thousands of free-thinkers to remain clever and engaged in our attempts to understand the world around us.

**Distribution.** Eastern United States east of the Mississippi River with southern limits in Florida.

**Remarks.** As it is likely that this species represents a cryptic species complex, measurements were only included from specimens exhibiting less than 2% COI divergence within the clade (those highlighted in red in Fig. 23 were excluded). This was done so measurements would remain useful if more species were diagnosed in the future.

*Testudacarus harrisi* O’Neill & Dowling, sp. n.

http://zoobank.org/EDE0FB53-D060-4879-8628-7DBBBF1749EA

**Type series. Holotype (1 ♀): North Carolina, USA:** 1 ♀ from Haywood County, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road near bridge 1.7km north of road to campground (35°38’45.00”N, 83°4’32.00”W), 20 September 2010, by IM Smith, IMS100150 (Specimen 146752 – DNA#2166); **Paratypes (12 ♀, 10 ♂): North Carolina, USA:** (allotype) 1 ♂ from Haywood County, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road near bridge 1.7km north of road to campground (35°38’45.00”N, 83°4’32.00”W), 20 September 2010, by IM Smith, IMS100150 (Specimen 146750 – DNA#2164); **Tennessee, USA:** 1 ♂ from Sevier County, Great Smoky Mountains National Park, Cosby Creek, Cosby Recreation Area beside Cosby Campground Road 0.3km from Route 321 (35°46’54.00”N, 83°13’2.00”W), 16 September 2010, by IM Smith, IMS100140; 2 ♀ and 2 ♂ from Sevier County, Great Smoky Mountains National Park, Bullhead Branch, Sugarlands Nature Trail off Route 441/71 (35°40’47.00”N, 83°31’52.00”W), 7 September 2009, by IM Smith, IMS090101; 1 ♀ from Sevier County, Great Smoky Mountains National Park, Bullhead Branch, Sugarlands Nature Trail off Route 441/71 (35°40’48.00”N, 83°31’53.00”W), 3 September 2009, by IM Smith, IMS090095; 1 ♂ from Blount County, Great Smoky Mountains National Park, Cades Cove, near parking lot for Abrams Falls Trail (35°35’26.00”N, 83°51’10.00”W), 17 September 2010, by IM Smith, IMS100143; 2 ♀ from Sevier Co, Great Smoky Mountains National Park, Bullhead Branch, Sugarlands Nature Trail off Route 441/71 (35°40’47.00”N, 83°31’51.00”W), 10 September 2010, by IM Smith, IMS100125; **North Carolina, USA:** 2 ♀ and 2 ♂ from Haywood County, Great Smoky Mountains National Park, Cataloochee Creek, beside Cataloochee Road 0.3km north of Cataloochee campground (35°38’1.00”N, 83°5’2.00”W), 6 September 2009, IMS090097; 2 ♀ and 1 ♂ from Haywood County, Great Smoky Mountains National Park, Big Creek, Water-ville Big Creek Picnic Area (35°44’59.00”N, 83°6’42.00”W), 16 September 2010, by
IM Smith, IMS100138; 1♀ and 1♂ from Haywood County, Great Smoky Mountains National Park, Cataloochee Creek, beside Mount Sterling Road near bridge 1.7 km north of road to campground (35°38'45.00"N, 83°4'32.00"W), 20 September 2010, by IM Smith, IMS100150; 1♂ from Yancey County, Pisgah National Forest, South Toe River, Lost Cove beside Toe River Road (Forest Road 472) 0.4 km east of Forest Road 2074 (35°45'0.00"N, 82°12'53.00"W), 9 September 2007, IM Smith, IMS070059; 1♀ from Haywood County, Great Smoky Mountains National Park, Rough Fork Creek, beside road to Nellie 0.3 km west of Pretty Hollow Gap Trailhead (35°37'31.00"N, 83°6'46.00"W), 20 September 2010, by IM Smith, IMS100148;

**Pennsylvania, USA:** 1♀ from Fayette County, State Game Lands #51, Dunbar Creek, off Furnace Hill Road east of Dunbar (39°57'50.00"N, 79°35'8.70"W), 10 August 2014, MJ Skvarla, MS14-0810-001.

**Type deposition.** Holotype (1♀), allotype (1♂) and ten paratypes (5♀, 5♂) deposited at Canadian National Collection; eleven paratypes (7♀, 4♂) at ACUA.

**Diagnosis.** These mites have violet to blue coloration over the majority of their anterio-lateral platelets while the rest of the complex have coloration restricted to the posterior half of the platelet.

**Description. Female (n=13) with characteristics of the genus with following specifications.**


Dorsum (Fig. 26) — [527–617 long; 420–482 wide] ovoid. Primary sclerotization violet to blue. Dorso-glandularia-4 [113–167 apart] lateral to [16–42] and around the anterior tips of the muscle scars. Platelets violet to blue or clear. Anterio-medial platelet [112–144 long; 70–94 wide] colorless rounded trapezoid noticeably smaller than anterio-lateral platelets. Anterio-lateral platelets [156–183 long; 74–86 wide] mostly violet to blue with anterio-most corner generally colorless; anterio-medial corner often with orange spot. Lateral platelets as follows: lateral-1 [38–50 long; 35–44 wide]; lateral-2 [103–133 long; 30–40 wide]; lateral-3 [29–45 long; 16–30 wide]; lateral-4 [90–119 long; 26–37 wide]; lateral-5 [47–64 long; 22–34 wide]; lateral-6 [65–90 long; 27–34 wide]; lateral-7 [56–69 long; 24–36 wide].

167–207 distance to excretory pore; 241–307 distance to caudad]. Distance to excretory pore [593–693].


Male (n=10) similar to female except for sexually dimorphic characters previously discussed and with following specifications.


Dorsum (Fig. 27) — [418–488 long; 312–380 wide]. Dorsal plate [340–402 long; 271–322 wide]. Dorso-glandularia-4 [89–129 apart] far anterior to [31–71] and lateral


Legs — total leg and podomere lengths as follows: Leg-I [419–451 total; trochanter 45–56; basifemur 70–77; telofemur 58–68; genu 74–82; tibia 81–90; tarsus 79–84]. Leg-II [429–472 total; trochanter 47–52; basifemur 69–77; telofemur 56–63;
Systematics of testudacarine torrent mites (Acari, Hydrachnidia, Torrenticolidae)...

Leg-III [491–540 total; trochanter 49–53; basifemur 70–86; telofemur 59–66; genu 89–98; tibia 107–120; tarsus 114–124]. Leg-IV [665–739 total; trochanter 74–90; basifemur 95–109; telofemur 95–108; genu 128–138; tibia 139–150; tarsus 131–145].

**Etymology.** Specific epithet after Samuel Benjamin Harris, the American author, philosopher, and co-founder of Project Reason. Sam Harris, more than any speaker and author, has challenged my (JCO) views and assumptions and kept me on my toes.

**Distribution.** Eastern United States east of the Mississippi river, with southern limits in Florida.

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*Testudacarus dennetti* O’Neill & Dowling, sp. n.

http://zoobank.org/C112BB32-CAD0-42A4-A757-5C318FAF067C

**Type series.** Holotype (1♀): Pennsylvania, USA: 1♀ from Fayette County, Ohiopyle State Park, Laurel Run, fishing access #2 off T798 (Meadow Run Rd) Ohiopyle State Park (39°50’58.00"N, 79°30’51.00"W), 10 August 2014, by MJ Skvarla, MS14-0810-005 (Specimen 143645 – DNA#2071); Paratypes (8♀, 7♂): Mississippi, USA: (allotype) 1♂ from Tishomingo County, Tishomingo State Park, Rocky Quarry Branch, beside road just outside park entrance (34°36’43.00"N, 88°12’4.00"W), 20 September 2009, by IM Smith, IMS090115 (Specimen 146784 – DNA#2202); 3♀ and 4♂ from Tishomingo County, Tishomingo State Park, Rocky Quarry Branch, beside road just outside park entrance (34°36’43.00"N, 88°12’4.00"W), 20 September 2009, by IM Smith, IMS090115; 2♀ and 2♂ from Tishomingo County, Tishomingo State Park, Rocky Quarry Branch, (34°36'”N, 88°11’W), 18 September 1991, by IM Smith, IMS910049; Pennsylvania, USA: 2♀ from Fayette County, State Game Lands #51, Dunbar Creek, off Furnace Hill Road east of Dunbar (39°57’50.00"N, 79°35’8.70"W), 10 August 2014, MJ Skvarla, MS14-0810-001; Alabama, USA: 1♀ from DeKalb County, Desoto State Park, beside Trail Y (Yellow) (34°29’N, 85°32’W), 26 September 1992, by IM Smith, IMS920053A.

**Type deposition.** Holotype (1♀), allotype (1♂), and six paratypes (3♀, 3♂) deposited at CNC; eight paratypes (5♀, 3♂) at ACUA.

**Diagnosis.** Both *Testudacarus dennetti* and *T. dawkinsi* have dorsal plates with uniform pores (unlike *T. hitchensi*) and anterio-lateral platelets with color restricted to the posterior half (unlike *T. harrisi*). However, they can be distinguished based on size. *Testudacarus dennetti* females and males have dorsal lengths less than 575 and 450 µm, respectively. *Testudacarus dawkinsi* females and males have dorsal lengths greater than 600 and 475 µm, respectively.

**Description.** Female (n=9) with characteristics of the genus with following specifications.


Male (n=7) similar to female except for sexually dimorphic characters previously discussed and with following specifications.


Legs — total leg and podomere lengths as follows: Leg-I [414–434 total; trochanter 47–54; basifemur 67–73; telofemur 55–62; genu 72–79; tibia 81–85; tarsus 79–85]. Leg-II [432–450 total; trochanter 48–54; basifemur 66–72; telofemur 54–61; genu 73–80; tibia 88–91; tarsus 96–99]. Leg-III [478–525 total; trochanter 49–58; basifemur 66–76; telofemur 58–64; genu 83–93; tibia 102–114; tarsus 114–126]. Leg-IV [658–685 total; trochanter 76–86; basifemur 85–103; telofemur 90–100; genu 124–130; tibia 140–141; tarsus 133–140].
Figure 29. Testudacarus dennetti male: (Left) dorsum; (Right) venter. Scale: 100 µm.

Etymology. Specific epithet *dennetti* after Daniel Clement Dennett III, the American philosopher, writer, and cognitive scientist. Dennett’s work has been the focus of many late night debates in close social circles just as he adds the necessary philosophical spice to the New Atheists.

Distribution. Eastern United States east of the Mississippi River, with southern limits in Florida.

*Testudacarus dawkinsi* O’Neill & Dowling, sp. n.
http://zoobank.org/4AC3753F-9E9D-4B38-9045-1BA094040E16

Type series. Holotype (1♀): New York, USA: 1♀ from Franklin County, Little Aldo Creek, Little Aldo Creek trail from Keese Mill Rd (44°25’32.00”N, 74°20’43.00”W), 19 July 2013, by AJ Radwell and C Milewski, AJR13-0719-205 (Specimen 141897 – DNA#1501); Paratypes (5♀, 9♂): Tennessee, USA: (allotype) 1♂ from Sevier County, Great Smoky Mountains National Park, Crosby Creek, Cosby Recreation Area beside Cosby Campground Road 0.3km from Route 321 (35°46’54.00”N, 83°13’2.00”W), 16 September 2010, by IM Smith, IMS100140 (Specimen 146744 – DNA#2156); 1♂ from Sevier County, Great Smoky Mountains National Park,
Bullhead Branch, Sugarlands Nature Trail off Route 441/71 (35°40’47.00"N, 83°31’51.00"W), 10 September 2010, by IM Smith, IMS100125; 2♀ and 1♂ from Sevier County, Great Smoky Mountains National Park, Crosby Creek, Cosby Recreation Area beside Cosby Campground Road 0.3km from Route 321 (35°46’54.00"N, 83°13’2.00"W), 16 September 2010, by IM Smith, IMS100140; 1♂ from Sevier County, Great Smoky Mountains National Park, Bullhead Branch, Sugarlands Nature Trail off Route 441/71 (35°40’47.00"N, 83°31’52.00"W), 24 September 2010, by IM Smith, IMS100158; 1♂ from Sevier County, Great Smoky Mountains National Park, Bullhead Branch, Sugarlands Nature Trail off Route 441/71 (35°40’48.00"N, 83°31’53.00"W), 3 September 2009, by IM Smith, IMS090095; 1♀ and 1♂ from Sevier County, Great Smoky Mountains National Park, Bullhead Branch, Sugarlands Nature Trail off Route 441/71 (35°40’47.00"N, 83°31’52.00"W), 7 September 2009, by IM Smith, IMS090101; 1♂ and 2♀ from Sevier County, Great Smoky Mountains National Park, Cosby Creek, beside road to Cosby Campground at Gabes Mountain Trailhead (35°45’27.00"N, 83°12’36.00"W), 19 September, by IM Smith, IMS050093A; Virginia, USA: 1♂ from Alleghany County, Simpson Creek, Longdale Furnace beside Route 850 2.2 km northeast of I-64 overpass (37°49’41.00"N, 79°39’30.00"W), 14 August 2008, by IM Smith, IMS080044; **North Carolina, USA:** 1♀ from Macon County, Rainbow Springs, beside Forest Road 67 4.4 km south of Standing Indian Campground (35°3’6.00"N, 83°30’45.00"W), 1 July 2006, by IM Smith, IMS060040.

**Type deposition.** Holotype (1♀), allotype (1♂), and six paratypes (3♀, 3♂) deposited at CNC; seven paratypes (2♀, 5♂) at ACUA.

**Diagnosis.** Both *Testudacarus dennetti* and *T. dawkinsi* have dorsal plates with uniform pores (unlike *T. hitchensi*) and anterio-lateral platelets with color restricted to the posterior half (unlike *T. harrisi*). However, they can be distinguished based on size. *Testudacarus dennetti* females and males have dorsal lengths less than 575 and 450 µm, respectively. *Testudacarus dawkinsi* females and males have dorsal lengths greater than 600 and 475 µm, respectively.

**Description. Female (n=6)** with characteristics of the genus with following specifications.


Figure 30. Testudacarus dawkinsi female: (Left) dorsum; (Right) venter. Scale: 100 µm.


Venter (Fig. 30) — [790–798; 510–534 wide] round to ovoid, fully sclerotized, and with anterior area of primary sclerotization [620–654 long] and posterior area of secondary sclerotization, violet to blue. Gnathosomal bay [77–84 dorsal length; 148–152 ventral length; 51–66 wide]. Coxal field [473–495 long; 330–368 wide]. Coxa-I [250–266 long; 100–114 midlength]. Coxa-II + III [119–125 distance to top of coxa-II; 188–195 distance to top of coxa-III; 350–370 distance to bottom of coxa-III; 229–245 total length]. Coxa-IV [325–343 distance to top; 490–501 distance to bottom; 150–162 total length; 122–137 width; 181–194 distance from gnathosomal bay; 69–90 distance from coxa-I; 191–208 distance to excretory pore; 293–304 distance to caudad]. Distance to excretory pore [682–707].

Legs — violet to blue. Total leg and podomere lengths as follows: Leg-I [487–500 total; trochanter 57–63; basifemur 84–86; telofemur 67–73; genu 90–94; tibia 94–99; tarsus 87–94]. Leg-II [532–548 total; trochanter 58–65; basifemur 84–89; telofemur 67–72; genu 94–99; tibia 107–113; tarsus 110–116]. Leg-III [599–629 total; tro-
Figure 31. *Testudacarus dawkinsi* male: (Left) dorsum; (Right) venter. Scale: 100 µm.


**Male (n=9)** similar to female except for sexually dimorphic characters previously discussed and with following specifications.


**Distribution.** Eastern United States east of the Mississippi River, with southern limits in Florida.

**Etymology.** Specific epithet *dawkinsi* after Clinton Richard Dawkins, the English evolutionary biologist and writer. Dawkins has proven repeatedly that one can change the world as a biologist by day and keep going as a free-thinker by night.

**Testudacarus americanus complex**

**Complex diagnosis.** These mites lack the four-segmented pedipalp of the *Debsacarus oribatoides*-like mites, the elongate body of the *Testudacarus elongatus*-like mites, and with the exception of *T. rollerae*, are much larger (female and male dorsal length usually more than 700 and 600 µm, respectively) than mites of the *T. minimus* and *T. hitchensi* complexes. *Testudacarus rollerae* have a larger (>140 µm) anterio-medial platelet that is more than or nearly twice as wide as long, while *T. minimus*-like mites have a smaller (<140 µm), more rounded anterio-medial platelet. These mites are present in western North America within and west of the Rocky Mountains, have very light to no coloration, have a large rectangular anterio-medial platelet, and comprise the following species: *T. kirkwoodae*, *T. americanus*, *T. hyporrhynchus*, *T. smithi*, and *T. rollerae*.

**Remarks.** Molecular data show strong support for five distinct clades (Fig. 32). Four clades exhibit less than 1.3% COI intraclade divergence, and all five clades exhibit greater than 9% divergence between clades. The fifth clade (pink in Fig. 32) exhibits 4.5% divergence within. However, only two specimens of this clade are available. One is teneral and badly damaged and therefore provides no characters for morphological diagnoses. More specimens should be collected and analyzed. Otherwise, all five clades have diagnostic morphological features that further warrant species designations.
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Figure 32. Testudacarus americanus complex molecular phylogeny: 28S and COI Bayesian analysis showing strong support for five distinct clades (●: >95% posterior probability); excluding pink clade, colored clades exhibit <1.3% divergence in COI within and >9% divergence between; pink exhibits 4.5% variation within; red specimen is a suspected species based on genetic data, but specimen is teneral and too badly damaged to diagnose; continuation of (C) lineage from Fig. 6.

Testudacarus americanus Marshall, 1943
http://zoobank.org/FE0E6228-D8AA-4063-A139-1C4A963454EB


Testudacarus americanus galloi: Habeeb 1969: 2

Type series. Holotype (1 ♀): California, USA: 1 ♂ from Santa Cruz County, Waddell Creek, 29–30 June 1933, by PR Needham, RM330008

Other material examined. Other (9 ♀, 8 ♂): Oregon, USA: 1 ♀ and 1 ♂ from Lincoln County, Siuslaw National Forest, Lord Creek, (44°14′24.00″N, 123°46′11.00″W), 8 August 2013, by JC O’Neill and WA Nelson, JNOW13-0808-002; 3 ♀ and 4 ♂ from Lane County, Cape Perpetua, Cape Perpetua Campground (44°16′51.00″N, 124°5′38.00″W), 15 September 2004, by IM Smith, IMS040077; 1 ♀ and 1 ♂ from Lane County, Rock Creek, Rock Creek Campground off Route 101 between Heceta Head and Yachats (44°11′6′.00″N, 124°6′34.00″W), 14 September 2004, by IM Smith, IMS040076; 1 ♀ from Lane County, Cape Creek, Cape Perpetua, Cape Perpetua Campground (44°16′51.00″N, 124°5′38.00″W), 24 June 2010, by IM
Smith, IMS100083; 1♀ and 1♂ from Curry County, Port Orford, beside road from Humbug Mountain State Park to McGribble Campground (Forest Road 5002) 5.3 km from Route 101 (42°42'11.00"N, 124°23'54.00"W), 25 June 1976, by IM Smith, IMS760161; 1♀ from Curry County, Port Orford, beside road from Humbug Mountain State Pk to McGribble Campground (Forest Road 5002) 4.6 km from Route 101 (42°42'3.00"N, 124°24'21.00"W), 17 June 2010, by IM Smith, IMS100070; 1♀ from Curry County, Siskiyou National Forest, North Fork of Foster Creek, beside Road #33 between Powers and Agness (42°39’N, 124°4’W), 2 July 1983, IMS830019; Washington, USA: 1♂ from Kittitas County, Wenatchee National Forest, Squawk Creek, (47°16’51.00"N, 120°41’53.00"W), 31 July 2013, by JC O’Neill, WA Nelson, JNOW13-0731-002.

**Type deposition.** Holotype (1♀) deposited at CNC.

**Diagnosis.** Resembling most *Testudacarus smithi*, these mites differ by shape, color, and several other characters. Most notably, *T. americanus* are elliptical and colorless to peach and have a small cheliceral fang (<33 µm) while *T. smithi* are rounded and are grey to colorless with large cheliceral fangs (>40 µm).

**Redescription.** Female (n=10) with characteristics of the genus with following specifications.


Venter (Fig. 33) — [973–1095 long; 620–731 wide] ovoid to oblong and colorless. Primary sclerotization [828–934 long]. Gnathosomal bay [83–100 dorsal length; 171–196 ventral length; 67–82 wide]. Coxal field [555–615 long; 393–478 wide] noticeably small in relation to the venter when compared to other Testudacarines. Coxa-I [279–326 long; 106–141 midlength]. Coxa-II + III [128–145 distance to top of coxa-II; 211–256 distance to top of coxa-III; 391–444 distance to bottom of coxa-III; 263–304 total length]. Coxa-IV [368–427 distance to top; 170–198 total length]. Genital field [375–438 distance to top; 570–641 distance to bottom; 188–203 total length; 157–170 width; 203–259 distance from gnathosomal bay; 94–118 distance
from coxa-I; 282–325 distance to excretory pore; 403–472 distance to caudad]. Eggs [182–200 long; 1–2 eggs]. Distance to excretory pore [880–964].


Male (n=8) similar to female except for sexually dimorphic characters previously discussed and with following specifications.


Dorsum (Fig. 34) — [678–755 long; 475–534 wide]. Dorsal plate [573–645 long; 405–463 wide]. Dorso-glandularia-4 [180–208 apart] lateral to [40–58] and well an-
Figure 34. *Testudacerus americanus* male: (Left) dorsum; (Right) venter. Scale: 100 µm.


Legs — total leg and podomere lengths as follows: Leg-I [501–560 total; trochanter 57–64; basifemur 85–99; telofemur 70–80; genu 90–102; tibia 101–113; tarsus 95–108]. Leg-II [508–567 total; trochanter 58–67; basifemur 88–96; telofe-
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Remarks. Having examined the type material, we suggest that *T. americanus galloi* Habeeb, 1969 is simply a teneral *T. americanus* that Habeeb confused for an “atypical” *T. americanus*.

*Testudacarus kirkwoodae* O’Neill & Dowling, sp. n.
http://zoobank.org/B46CE9B8-65BC-4F12-B8C2-791C23CDB4BA

**Type series.** Holotype (1 ♀) Oregon, USA: 1 ♀ from Douglas County, Rouge River National Forest, Muir Creek, (43°2'53.00"N, 122°21'4.00"W), 12 August 2013, by JC O’Neill and WA Nelson, JNOW13-0812-004 (Specimen 141885 – DNA#1791);

Other examined but not measured (1 ♀): Washington, USA: 1 ♀ from Snohomish County, Mount Baker National Forest, tributary of South Fork of Sauk River, (48°1'40.00"N, 121°26'24.00"W), 28 July 2013, JC O’Neill and WA Nelson, JNOW13-0728-003

**Type deposition.** Holotype (1 ♀) deposited at CNC.

**Diagnosis.** These are the largest known testudacarines and also differ further from their complex in having a smaller, more rounded anterio-medial platelet.

**Description.** Female (n=1) with characteristics of genus with following specifications.

- **Dorsum (Fig. 35) — [918 long; 645 wide] ovoid to oblong. Dorsal plate [758 long; 566 wide]. Primary sclerotization [603 long] light pink to colorless. Dorso-glandularia-4 [232 apart] lateral to [63] and around muscle scar midline. Platelets colorless. Anterio-medial platelet [201 long; 123 wide]. Anterio-lateral platelets [237 long; 134 wide]. Lateral platelets as follows: lateral-1 [65 long; 55 wide]; lateral-2 [173 long; 46 wide]; lateral-3 [67 long; 24 wide]; lateral-4 [173 long; 46 wide]; lateral-5 [91 long; 47 wide]; lateral-6 [149 long; 54 wide]; lateral-7 [93 long; 46 wide].
- **Venter (Fig. 35) — [1045 long; 853 wide] round to ovoid and colorless. Primary sclerotization [752 long]. Gnathosomal bay [123 dorsal length; 151 ventral length; 69 wide]. Coxal field [551 long; 484 wide] proportionally small compared to venter. Coxa-I [294 long; 144 midlength]. Coxa-II + III [114 distance to top of coxa-II; 202
distance to top of coxa-III; 393 distance to bottom of coxa-III; 279 total length].
Coxa-IV [376 distance to top; 175 total length]. Genital field [373 distance to top;
578 distance to bottom; 205 total length; 69 width; 222 distance from gnathosomal
bay; 78 distance from coxa-I; 272 distance to excretory pore; 467 distance to caudal].
Distance to excretory pore [850].

Legs — colorless. Total leg and podomere lengths as follows: Leg-I [620 total;
trochanter 70; basifemur 119; telofemur 89; genu 101; tibia 119; tarsus 121]. Leg-II
[681 total; trochanter 77; basifemur 113; telofemur 90; genu 117; tibia 145; tarsus
140]. Leg-III [756 total; trochanter 70; basifemur 116; telofemur 93; genu 144; tibia
165; tarsus 167]. Leg-IV [1081 total; trochanter 136; basifemur 148; telofemur 159;
genu 207; tibia 224; tarsus 208].

Male (n=0) unknown.

Etymology. Specific epithet kirkwoodae after my (JCO) mother’s maiden name. It
was her bringing me to the leafcutter ant exhibit at our local science center that helped
me become interested in biology as a child, and her endless support and advice that
helped me finish my education.

Remarks. As it is likely that this species represents a cryptic species complex (with
4.5% COI divergence between the two available specimens), measurements were in-
cluded from only one specimen (the other highlighted in red in Fig. 32 was excluded). This was done so measurements would remain useful if more species were diagnosed in the future. Measurements were also not included from the other specimen because it is teneral and badly damaged and would therefore prove poor for any description. While more than a single specimen is certainly desired for new descriptions, the included specimen has unique morphological characters such as its large size (it is larger than any other testudacarine, including species from Asia), and has strong support as a unique clade using COI. Therefore, this single specimen is unique enough that we are comfortable describing it.

**Distribution.** Only one specimen know from Douglas County, Oregon.

*Testudacarus hyporhynchus* O’Neill & Dowling, sp. n.
http://zoobank.org/371AF7BE-204F-4CA7-8CE4-34EF7647B751

**Type series.** Holotype (1♀): California, USA: 1♀ Humboldt County, Willow Creek, Willow Creek Campground off Rt. 299 (40°54′17.00″N, 123°42′21.00″W), 14 June 2010, by IM Smith, IMS100065 (Specimen 146762 – DNA#2177); Para-type (1♀, 2♂): California, USA: (allotype) 1♂ Humboldt County, Willow Creek, Willow Creek Campground off Rt. 299 (40°54′17.00″N, 123°42′21.00″W), 14 June 2010, by IM Smith, IMS100065 (Specimen 146763 – DNA#2178); 1♂ Humboldt County, Willow Creek Campground off Rt. 299 (40°54′17.00″N, 123°42′21.00″W), 14 June 2010, by IM Smith, IMS100065; Oregon, USA: 1♀ from Curry County, Port Orford, beside Elk River Road 9.0 km east of Elk River Fish Hatchery (42°42′22.00″N, 124°20′28.00″W), 22 June 2010, by IM Smith, IMS100080.

**Type deposition.** Holotype (1♀) and allotype (1♂) deposited at CNC; two paratypes (1♀, 1♂) at ACUA.

**Diagnosis.** These mites differ from the rest of the complex in having a dorsally “covered” gnathosomal bay (short dorsal gnathosomal bay length) and an elongate gnathosoma with a long rostrum that extends below the gnathosoma ventral surface.

**Description. Female (n=2)** with characteristics of genus with following specifications.


Figure 36. *Testudacarus hyporhynchus* sp. n. gnathosoma (generalized).

Wide]. Lateral platelets as follows: lateral-1 [66–83 long; 56–59 wide]; lateral-2 [156–188 long; 47–51 wide]; lateral-3 [60–84 long; 29–30 wide]; lateral-4 [139–150 long; 35–45 wide]; lateral-5 [79–93 long; 41–42 wide]; lateral-6 [129–144 long; 39–44 wide]; lateral-7 [76–88 long; 35–41 wide].


Male (n=2) similar to female except for sexually dimorphic characters previously discussed and with following specifications.


**Etymology.** Specific epithet hyporhynchus (hypo-, G. under; rhynchus, G. snout) refers to the long rostrum that extends below the ventral surface of the gnathosoma.

**Distribution.** Humboldt County, California and Curry County, Oregon.

*Testudacarus smithi* O’Neill & Dowling, sp. n.
http://zoobank.org/870A0931-9FDB-4293-B6E9-2AB5BAA733C5

**Type series.** Holotype (1♀): British Columbia, Canada: 1♀ from Vancouver Island, spring run, Lake Cowichan, beside North Shore Road 1.7 km north of town (48°49’29.00”N, 124°4’2.00”W), 1 July 2010, by IM Smith, IMS100091 (Speci-
men 146769 – DNA#2184); **Paratypes (10♀, 12♂):** British Columbia, Canada: (allotype) 1♂ from Vancouver Island, spring run, Lake Cowichan, beside North Shore Road 1.7 km north of town (48°49’29.00”N, 124°4’2.00”W), 1 July 2010, by IM Smith, IMS100091 (Specimen 146770 – DNA#2185); 2♂ from Vancouver Island, spring run, Lake Cowichan, beside North Shore Road 1.7 km north of town (48°49’29.00”N, 124°4’2.00”W), 1 July 2010, by IM Smith, IMS100091; 3♀ and 2♂ from Vancouver Island, Lake Cowichan, spring run, beside North Shore Road 1.7 km north of town (48°49’29.00”N, 124°4’13.00”W), 11 June 1979, by IM Smith, IMS790013A; 3♀ and 3♂ from Vancouver Island, Lake Cowichan, spring run, beside South Shore Road 2.3 km north of town (48°48’25.00”N, 124°5’13.00”W), 7 July 1976, by IM Smith, IMS760194; 3♂ and 2♂ from Vancouver Island, Port Alberni, beside road to Mount Arrowsmith Ski Area 11.6 km from Highway 4 (49°12’50.00”N, 124°36’18.00”W), 19 September 2004, by IM Smith, IMS040084A; 1♀ and 1♂ from Vancouver Island, Lake Cowichan, spring run, beside South Shore Road 2.3 km north of town (48°48’25.00”N, 124°5’13.00”W), 31 July 1979, by IM Smith, IMS790056; 1♂ from Vancouver Island, Lake Cowichan, spring run, beside South Shore Road 2.3 km north of town (48°48’25.00”N, 124°5’13.00”W), 26 July 1985, by IM Smith, IMS850122A.

**Type deposition.** Holotype (1♀), allotype (1♂), and eight paratypes (4♀, 4♂) deposited at CNC; thirteen paratypes (6♀, 7♂) at ACUA.

**Diagnosis.** Resembling most *Testudacarus americanus*, these mites differ by shape, color, and several other characters. Most notably, *T. americanus* are elliptical and colorless to peach and have a small cheliceral fang (<33 µm) while *T. smithi* are rounded and are grey to colorless with large cheliceral fangs (>40 µm).

**Description. Female (n=10)** with characteristics of genus with following specifications.


**Venter (Fig. 39) —** [955–1047; 671–816 wide] round to ovoid and colorless. **Primary sclerotization** [742–814 long]. **Gnathosomal bay** [87–130 dorsal length;


**Male (n=12)** similar to female except for sexually dimorphic characters previously discussed and with following specifications.


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**Figure 39.** *Testudacarus smithi* female: (Left) dorsum; (Right) venter. Scale: 100 µm.
Figure 40. *Testudacarus smithi* male: *(Left)* dorsum; *(Right)* venter. Scale: 100 µm.


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wide]. Distance to excretory pore [790–874]. Excretory pore characteristically well
separated from line of secondary sclerotization.

Legs — total leg and podomere lengths as follows: Leg-I [617–679 total; tro-
chanter 73–80; basifemur 110–122; telofemur 84–97; genu 116–129; tibia 118–138;
tarsus 107–123]. Leg-II [664–743 total; trochanter 74–84; basifemur 110–125; tel-
ofemur 85–103; genu 118–137; tibia 134–157; tarsus 131–148]. Leg-III [753–841
total; trochanter 76–83; basifemur 111–133; telofemur 89–106; genu 138–158; tibia
163–186; tarsus 155–186]. Leg-IV [952–1098 total; trochanter 111–131; basifemur
136–154; telofemur 139–161; genu 189–217; tibia 189–238; tarsus 176–210].

**Etymology.** Specific epithet *smithi* after Dr Ian Smith, the Canadian water mite
researcher who collected the specimens needed for this description. Dr Smith has ad-
vanced water mite research in North America more than anyone else and without him,
this work would have been impossible.

**Distribution.** British Columbia, Canada.

*Testudacarus rollerae* O’Neill & Dowling, sp. n.
http://zoobank.org/74EBEF64-B599-45A8-B79E-6A07D1DA05A8

**Type series.** Holotype (1♀): California, USA: 1♀ from Nevada County, Tahoe
National Forest, Bear River, at Sierra Discovery day use area upstream from bridge
(39°18'35.00"N, 120°39'56.00"W), 26 August 2013, by JR Fisher, JRF13-0826-001
(Specimen 146725 – DNA# 2135); Paratypes (2♀, 2♂): California, USA: (allotype)
1♂ (allotype) from Nevada County, Tahoe National Forest, Bear River, at Sierra Discov-
ery day use area upstream from bridge (39°18'35.00"N, 120°39'56.00"W), 26 August
2013, by JR Fisher, JRF13-0826-001 (Specimen 146724 – DNA# 2134); 1♀ from
Nevada County, Tahoe National Forest, Bear River, at Sierra Discovery day use area up-
stream from bridge (39°18'35.00"N, 120°39'56.00"W), 26 August 2013, by JR Fisher,
JRF13-0826-001; 1♀ and 1♂ from Calaveras County, Calaveras Big Trees State Park,
Big Trees River, (38°16'N, 120°16’W), 12 June 1976, by IM Smith, IMS760099; 1♂
from Mendocino County, Navarro River, Paul M. Dimmick Recreation Area beside
Route 128 (39°10'N, 123°38’W), 29 September 1993, by IM Smith, IMS9300026A.

**Type deposition.** Holotype (1♀) and allotype (1♂) deposited at CNC; four para-
types (2♀, 2♂) at ACUA.

**Diagnosis.** These mites are smaller and more colorful than other species in the
complex and therefore resemble most the *T. minimus*-like mites; however, mites of the
*T. minimus* complex are even smaller and have a smaller (<140 µm, and far less than
twice as wide as long), more rounded anterio-medial platelet, while these mites have
a larger (>140 µm, and more than or nearly twice as wide as long) more rectangular
anterio-medial platelet.

**Description. Female (n=3)** with characteristics of genus with following specifications.

Gnathosoma — Subcapitulum [176–188 ventral length; 105–107 dorsal length;
100–103 tall] elliptical ovoid with short rostrum and colorless. Chelicerae [145–152


Figure 41. *Testudacarus rollerae* female: (Left) dorsum; (Right) venter. Scale: 100 μm.
Genital field [347–374 distance to top; 500–539 distance to bottom; 153–165 total length; 130–139 width; 193–210 distance from gnathosomal bay; 79–87 distance from coxa-I; 199–231 distance to excretory pore; 286–345 distance to caudad]. Eggs [165–178 long; 1 egg]. Distance to excretory pore [699–770].


Male (n=3) similar to female except for sexually dimorphic characters previously discussed and with following specifications.


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Figure 42. *Testudacarus rollerae* male: (Left) dorsum; (Right) venter. Scale: 100 µm.

**Etymology.** Specific epithet *rollerae* after Elizabeth Ashley Roller, my (JCO) life partner.

**Distribution.** Reported from only two counties (Mendocino and Nevada) in California.

*Testudacarus elongatus* complex

**Complex diagnosis.** Unlike all other Testudacarinae, members of this complex have an elongate idiosoma. In contrast to most *T. minimus*- and *T. hitchensi*-like mites, these mites are colorless and much larger (female and male dorsal length greater than 700 and 600 µm, respectively). These mites are found in western North America west of the Rocky Mountains. This complex comprises three species: *T. elongatus*, *T. oblongatus*, and *T. rectangulatus*.

**Remarks.** Combined molecular, distributional, and morphological data support three distinct clades within the *T. elongatus* complex (Fig. 43). All three clades exhibit less than 2.4% COI intra-clade divergence and greater than 3.3% divergence between clades. Intra-clade divergence of 2.4%, as seen with *T. oblongatus*, is not unexpected for a species exhibiting a large geographic range (British Columbia to California); how-
Figure 43. Testudacarus elongatus complex molecular phylogeny: 28S and COI Bayesian analysis showing strong support at least three distinct clades (●: >95% posterior probability); colored clades exhibit <2.4% divergence in COI within and >3.3% divergence between; divergence of the two basal clades >9%; continuation of (D) lineage from Fig. 43.

ever, a percent difference as high as 3.3% between two close localities (Mason and Snohomish County, between T. rectangulatus and T. elongatus) suggests separate species. Interestingly, COI divergence of more than 9% between the two sister clades (T. oblongatus and T. elongatus/T. rectangulatus) does not seem to produce high amounts of morphological diversity within this complex. Therefore, the morphological variation and geographic variation found between T. elongatus and T. rectangulatus provide enough evidence for us to hypothesize two species, even if one is from a single specimen. Potentially, there is a coastal species (T. oblongatus), a species within and east of the cascade (T. elongatus), and a species from the Olympic Mountains (T. rectangulatus).

Testudacarus elongatus O’Neill & Dowling, sp. n.
http://zoobank.org/4A0801F6-C137-48DD-B16C-333E68F81A56

Type series. Holotype (1♀): Washington, USA: 1♀ from Okanogan County, Okanogan National Forest, Early Winters Creek, (48°35’55.00"N, 120°35’20.00"W), 29 July 2013, by JC O’Neill and WA Nelson, JNOW13-0729-004 (Specimen 138495 – DNA#1522); Paratypes (7♀, 4♂): Washington, USA: (allotype) 1♂ from Okanogan County, Okanogan National Forest, Early Winters Creek, (48°35’55.00"N, 120°35’20.00"W), 29 July 2013, by JC O’Neill and WA Nelson, JNOW13-0729-004 (Specimen 141889 – DNA#1797); 1♀ from Whatcom County, Mount Baker National Forest, Porcupine Creek, (48°31’51.00"N, 120°44’42.00"W), 29 July 2013, by JC O’Neill and WA Nelson, JNOW13-0729-003; 2♀ and 2♂ from Okanogan County, Okanogan National Forest, Early Winters Creek, (48°35’55.00"N,
Systematics of testudacarine torrent mites (Acari, Hydrachnidia, Torrenticolidae)...


**Type deposition.** Holotype (1 ♀), allotype (1 ♂), and four paratypes (3 ♀, 1 ♂) deposited at CNC; six paratypes (4 ♀, 2 ♂) at ACUA.

**Diagnosis.** Since morphological variation is limited, a combination of morphology and distribution is best used to diagnose members of the complex. These mites occur in Washington within and east of the Cascade Mountains, while *T. rectangulatus* occur in the Olympic Mountains, and *T. oblongatus* occur along the western Coast of Washington, Oregon, California, and British Columbia. Additionally, both *T. rectangulatus* and these mites differ from *T. oblongatus* in having more robust lateral platelets; most notably, lateral-platelet-4 tends to be larger in these two species than *T. oblongatus*, and is in direct or near direct contact with lateral-platelet-2. Reversely, *T. oblongatus* generally have less robust platelets and a smaller lateral-platelet-4 that has a noticeable gap between it and lateral-platelet-2. Limited specimens were found of *T. elongatus* and *T. rectangulatus*, but *T. rectangulatus* appear to have leg and pedipalp measurements roughly 10% larger than *T. elongatus* even between individuals of similar idiosoma size. More data is needed to better diagnose these species.

**Description.** Female (n=8) with characteristics of genus with following specifications.


Venter (Fig. 44) — [947–1051; 536–682 wide] oblong. Primary sclerotization [798–880 long]. Gnathosomal bay [86–108 dorsal length; 138–178 ventral length; 63–95 wide]. Coxal field [185–198 long; 366–479 wide]. Coxa-I [260–307 long; 118–133 midlength]. Coxa-II + III [95–134 distance to top of coxa-II; 190–221 distance to
Figure 44. *Testudacarus elongatus* female: (Left) dorsum; (Right) venter. Scale: 100 µm.

top of coxa-III; 383–432 distance to bottom of coxa-III; 273–314 total length]. Coxa-
IV [362–394 distance to top; 182–207 total length]. Genital field [383–419 distance
to top; 568–614 distance to bottom; 185–198 total length; 140–166 width; 221–262
distance from gnathosomal bay; 102–132 distance from coxa-I; 265–298 distance to
excretory pore; 377–443 distance to caudad]. Eggs [270 long; 1–2 eggs]. Distance to
excretory pore [846–910].

Legs — colorless. Total leg and podomere lengths as follows: Leg-I [561–614 total;
trochanter 63–66; basifemur 101–114; telofemur 79–93; genu 103–116; tibia 110–
127; tarsus 96–109]. Leg-II [559–623 total; trochanter 56–65; basifemur 96–120;
telofemur 75–88; genu 103–116; tibia 120–128; tarsus 107–120]. Leg-III [630–703
total; trochanter 60–80; basifemur 97–116; telofemur 79–96; genu 116–139; tibia
136–152; tarsus 129–140]. Leg-IV [863–920 total; trochanter 98–109; basifemur
126–140; telofemur 130–140; genu 172–189; tibia 183–194; tarsus 152–167].

**Male** (n=4) similar to female except for sexually dimorphic characters previously
discussed and with following specifications.


Venter (Fig. 45) — [830–890 long; 497–578 wide]. Primary sclerotization [764–812 long]. Gnathosomal bay [72–85 dorsal length; 137–157 ventral length; 71–86 wide]. Coxal field [530–564 long; 372–390 wide]. Coxa-I [233–268 long; 96–112 midlength]. Coxa-II + III [95–114 distance to top of coxa-II; 180–201 distance to top

Legs — total leg and podomere lengths as follows: Leg-I [531–558 total; trochanter 54–65; basifemur 95–99; telofemur 75–80; genu 98–104; tibia 105–111; tarsus 100–105]. Leg-II [549–572 total; trochanter 59–65; basifemur 93–101; telofemur 73–79; genu 98–105; tibia 112–120; tarsus 100–109]. Leg-III [577–610 total; trochanter 64–69; basifemur 93–105; telofemur 75–84; genu 106–119; tibia 122–135; tarsus 117–131]. Leg-IV [765–812 total; trochanter 91–100; basifemur 110–120; telofemur 115–121; genu 153–171; tibia 152–168; tarsus 140–152].

Etymology. Specific epithet elongatus (elong-, L. extend) refers to the elongate idiosoma of adults.


_Testudacarus rectangulatus_ O’Neill & Dowling, sp. n.

http://zoobank.org/A7CA6FCE-2D33-482B-AF90-7C93964F5D71

Type series. Holotype (1♂): Washington, USA: 1♂ from Mason County, Olympic National Forest, Cabin Creek, by Hamma Hamma River (47°35’44.00"N, 123°7’39.00"W), 22 July 2013, by JC O’Neill and WA Nelson, JNOW13-0722-004 (Specimen 138494 – DNA#1521)

Type deposition. Holotype (1♂) deposited at CNC

Diagnosis. Since morphological variation is limited, a combination of morphology and distribution is best used to diagnosed members of the complex. These mites occur in the Olympic Mountains, while _T. elongatus_ occur in Washington within and east of the Cascade Mountains, and _T. oblongatus_ occur along the western Coast of Washington, Oregon, California, and British Columbia. Additionally, both _T. elongatus_ and these mites differ from _T. oblongatus_ in having more robust lateral platelets; most notably, lateral-platelet-4 tends to be larger in these two species than _T. oblongatus_, and is in direct or near direct contact with lateral-platelet-2. Reversely, _T. oblongatus_ generally have less robust platelets and a smaller lateral-platelet-4 that has a noticeable gap between it and lateral-platelet-2. Limited specimens were found of _T. elongatus_ and _T. rectangulatus_, but _T. rectangulatus_ appear to have leg and pedipalp measurements roughly 10% larger than _T. elongatus_ even between individuals of similar idiosoma size. More data is needed to better diagnose these species.

Description. Female (n=0) unknown.

Male (n=1) with characteristic of genus with following specifications.

Figure 46. *Testudacarus rectangulatus* male: (Left) dorsum; (Right) venter. Scale: 100 μm.


Dorsum (Fig. 46) — [773 long; 495 wide] oblong and colorless. Dorsal plate [649 long; 413 wide]. Dorsal plate lateral to [41] and anterior to [63] muscle scars. Platelets colorless. Antero-medial platelet [183 long; 108 wide] trapeziform to nearly triangular (posterior margin strongly shortened). Antero-lateral platelets [216 long; 114 wide] near rectangular. Lateral platelets as follows: lateral-1 [40 long; 45 wide]; lateral-2 [161 long; 41 wide]; lateral-3 [39 long; 23 wide]; lateral-4 [165 long; 40 wide]; lateral-5 [55 long; 34 wide]; lateral-6 [112 long; 49 wide]; lateral-7 [69 long; 37 wide].

Venter (Fig. 46) — [929 long; 492 wide] oblong. Primary sclerotization [855 long]. Gnathosomal bay [83 dorsal length; 162 ventral length; 89 wide]. Coxa field [577 long; 390 wide]. Coxa-I [278 long; 116 midlength]. Coxa-II + III [122 distance to top of coxa-II; 203 distance to top of coxa-III; 439 distance to bottom of coxa-III;
317 total length]. Coxa-IV [375 distance to top; 201 total length]. Genital field [461
distance to top; 647 distance to bottom; 186 total length; 133 width; 299 distance
from gnathosomal bay; 183 distance from coxa-I; 208 distance to excretory pore; 282
distance to caudad]. Genital skeleton [250 long]. Distance to excretory pore [855].

Legs — colorless. Total leg and podomere lengths as follows: Leg-I [603 total;
trochanter 61; basifemur 103; telofemur 89; genu 116; tibia 124; tarsus 108]. Leg-II
[610 total; trochanter 63; basifemur 101; telofemur 85; genu 115; tibia 126; tarsus 120]. Leg-III [674 total; trochanter 63; basifemur 110; telofemur 86; genu 130; tibia 145; tarsus 137]. Leg-IV [870 total; trochanter 87; basifemur 123; telofemur 130;
genu 179; tibia 189; tarsus 160].

**Etymology.** Specific epithet *rectangulatus* (*rectangulum*, L. straight angle) refers to
the boxy, elongate idiosoma of adults.

**Distribution.** One specimen found in Mason County in the Olympic Mountains,
Washington.

*Testudacarus oblongatus* O’Neill & Dowling, sp. n.
http://zoobank.org/411D6BD4-3740-4FE0-A7BB-30733BF28851

**Type series.** Holotype (1♀): Oregon, USA: 1♀ from Curry County, Siskiyou Na-
tional Forest, confluence of tributary and Wheeler Creek, off NF 1205 (42°4'42.00"N,
124°8'53.00"W), by JR Fisher, JRF13-0814-004 (Specimen 146728 – DNA#2138);
Paratypes (11♀, 9♂): British Columbia, Canada: (allotype) 1♂ from Vancouver Is-
land, beside Harris Creek Mainline 5 km west of Old Hillcrest Gate (26 km west
of Mesachie Lake) (48°40'7.00"N, 124°13'20.00"W), 3 July 2010, by IM Smith,
IMS100095 (Specimen 146776 – DNA#2192); 2♀ and 1♂ from Vancouver Island,
beside Harris Creek Mainline 5 km west of Old Hillcrest Gate (26 km west of Mesachie
Lake) (48°40'7.00"N, 124°13'20.00"W), 3 July 2010, by IM Smith, IMS100095; 3♀
and 1♂ from Vancouver Island, beside Highway 4 16.6 km east of road to Ucluelet (Pa-
cific Rim Road) (49°9'N, 125°54'W), 18-19 July 1979, by IM Smith, IMS790047; 1♀
and 3♂ from Bonanza Pass Walker Creek Picnic Area beside Highway 3 between Grand
Forks and Castlegar (49°10'N, 118°5'W), 20 July 1988, by IM Smith, IMS880034;
1♂ from Vancouver Island, Honeymoon Bay Wildflower Reserve, (48°49'38.00"N,
124°12'10.00"W), 19 June 1979, by IM Smith, IMS790023A; 1♀ from Vancouver
Island, beside Harris Creek Mainline 5 km west of Old Hillcrest Gate (26 km west
of Mesachie Lake) (48°40'6.00"N, 124°13'19.00"W), 3 July 2010, by IM Smith, IMS100097;
2♀ from Vancouver Island, beside Harris Creek Mainline 5 km west of
Old Hillcrest Gate (26 km west of Mesachie Lake) (48°40'6.00"N, 124°13'16.00"W),
10 July 1988, by IM Smith, IMS880007; California, USA: 1♂ from Monterey Coun-
ty, Los Padres National Forest, Lucia, beside Ferguson-Nacimiento Road 5.6 km east
of Route 1 (36°0'3.00"N, 121°28'31.00"W), 3 June 2010, by IM Smith, IMS100048;
1♂ from Trinity County, Shasta-Trinity National Forest, beside Route 36 6.2 km
west of Forest Glen Station Campground (40°22'57.00"N, 123°23'26.00"W), 11 June
Systematics of testudacarine torrent mites (Acari, Hydrachnidia, Torrenticolidae)...

2010, by IM Smith, IMS100061; 1 ♀ from Trinity County, Shasta-Trinity National Forest, beside Route 36 7 km west of Forest Glen Station Campground (40°23'5.00"N, 123°23'57.00"W), 11 June 2010, by IM Smith, IMS100062; Oregon, USA: 1 ♀ from Curry County, Siskiyou National Forest, confluence of tributary and Wheeler Creek, off NF 1205 (42°4'42.00"N, 124°8'53.00"W), by JR Fisher, JRF13-0814-004.

**Type deposition.** Holotype (1 ♀), allotype (1 ♂), and ten paratypes (6 ♀, 4 ♂) deposited at CNC; nine paratypes (5 ♀, 4 ♂) at ACUA.

**Diagnosis.** Since morphological variation is limited, a combination of morphology and distribution is best used to diagnosed members of the complex. These mites occur along the western coast of Washington, Oregon, California, and British Columbia, while *T. elongatus* occur in Washington within and east of the Cascade Mountains, and *T. rectangulatus* occur in the Olympic Mountains. These mites differ from others in the complex in having less robust platelets and a smaller lateral-platelet-4 that has a noticeable gap between it and lateral-platelet-2. Reversely lateral-platelet-4 tends to be larger in the other two species of the complex than *T. oblongatus*, and is in direct or near direct contact with lateral-platelet-2.

**Description. Female (n=11)** with characteristics of genus with following specifications.


**Male (n=9)** similar to female except for sexually dimorphic characters previously discussed and with following specifications.


**Figure 47.** *Testudacarus oblongatus* female: (Left) dorsum; (Right) venter. Scale: 100 µm.


Legs — total leg and podomere lengths as follows: Leg-I [526–617 total; trochant er 57–69; basifemur 90–103; telofemur 75–90; genu 100–116; tibia 105–124; tarsus 97–116]. Leg-II [536–629 total; trochanter 59–70; basifemur 87–106; telofemur 73–86; genu 100–117; tibia 106–134; tarsus 104–123]. Leg-III [589–691 total; trochanter 60–73; basifemur 89–111; telofemur 71–90; genu 115–130; tibia 123–151; tarsus 128–147]. Leg-IV [810–878 total; trochanter 92–101; basifemur 112–125; telofemur 114–127; genu 144–177; tibia 158–188; tarsus 143–168]

**Etymology.** Specific epithet *oblungatus* (*oblung-,* L. rather long) referring to the oblong idiosoma.

**Distribution.** West coast of British Columbia, Washington, Oregon, and California.

**Asian species**

*Testudacarus tripeltatus* Walter, 1928

http://zoobank.org/3871B946-C254-4A5E-81A2-2B4E45E5A47F


**Type series.** Holotype (1♀): Himachal Pradesh, India: (1♀) from Kangra Valley, Upper Dharamsala, Bhagsunath, June 4th 1926, by Dr Hora.

**Type deposition.** Holotype (1♀) at Naturhistorisches Museum Basel, Switzerland.

**Diagnosis.** *Testudacarus tripeltatus* can be differentiated from all other Asian species by distribution (India, Java, and Bhutan) and large size (dorsal length >700 µm). More research and updated descriptions are needed for a better diagnosis.

**Distribution.** India (Walter 1928), Java (Walter 1929), and Bhutan (Pešić and Smit 2007).

*Testudacarus japonicus* Imamura, 1955

http://zoobank.org/F3B6396B-0BEB-475E-B83D-83B6AF9C2D71

Systematics of testudacarine torrent mites (Acari, Hydrachnidia, Torrenticolidae)...


**Type series.** **Holotype (1♂): Shizuoka, Japan:** brook connected with a stream in Takékura, Mishima, Shizuoka, Japan, 15 May 1953, by T. Imamura.

The types were not examined for this publication.

**Type deposition.** Holotype (1♂) at Taiji Imamura Collection at Ibaraki Nature Museum, Japan.

Holotype loans are not available from Ibaraki Nature Museum. The museum provided a low-magnification photograph through e-mail, though permission to print the photograph was not obtained.

**Diagnosis.** These mites differ from all other Testudacarinae by distribution (Japan), and from *T. tripeltatus* by small size (dorsal length <700 µm). *Testudacarus japonicus* may be conspecific with *T. okadai*. More research and updated descriptions are needed for a better diagnosis.

**Distribution.** Takékura, Japan (Imamura 1955).

**Remarks.** It is reasonable to assume Imamura (1955) had no knowledge of Habeeb (1954) because he never mentions *T. vulgaris* and there are inaccuracies in his description that could have been prevented if he had. Firstly, his “female” type specimen is almost certainly a male as “the genital area [is] relatively more to the posterior than in [females] and the two [dorsal muscle scars]… are located posterior to the [glandularia]” (Habeeb 1954). Furthermore, in his remarks he states the “Japanese specimen resembles most the Indian species,” which with more current information is unlikely. At the time, *T. japonicus* would have been most similar in size, color, and shape to either *T. vulgaris* or *T. minimus*, not *T. tripeltatus*. Most importantly, the *T. japonicus* type is almost certainly male and therefore shares little morphology with the female *T. tripeltatus*. Therefore, the distinctions Imamura (1955) offers that *T. japonicus* are “different from [T. tripeltatus] in the anterior tips of the first [coxae], [pedi]palps, situations of [coxae] and genital organ” are unhelpful (Imamura 1955). He is referring to sexual dimorphism and comparing only the two most disparate species available to him.

*Testudacarus okadai* Imamura, 1976
http://zoobank.org/B66EFC5E-2C6A-448A-91E8-C5B1D7922654


**Type series.** **Holotype (1♀): Tichigi, Japan:** Onisawa, Shôbuga-Hama, Nikkô National Park, 13 May 1974, by Y. Okada.; **Allotype (1♂): Tichigi, Japan:** Onisawa, Shôbuga-Hama, Nikkô National Park, 13 May 1974, by Y. Okada.

The types were not examined for this publication.
Type deposition. Holotype (1♀) and allotype (1♂?) at Taiji Imamura Collection at Ibaraki Nature Museum, Japan.

Holotype loans are not available from Ibaraki Nature Museum. The museum provided a low-magnification photograph through e-mail, though permission to print the photograph was not obtained.

Diagnosis. These mites differ from all other Asian Testudacarinae by distribution (Japan), and from *T. tripeltatus* by small size (dorsal length <700 µm). *Testudacarus okadai* may be conspecific with *T. japonicus*. More research and updated descriptions are needed for a better diagnosis.

Distribution. Throughout Honshu, Japan (Imamura 1980).

Discussion. A drawing of the “male” dorsum is left out of the *T. okadai* description. This is of the utmost importance because the sex of the “male” specimen is in question. The positioning of the genital field in relation to coxae-IV and the short coxae-II+III midline is typical of female testudacarines, but the coxal field size in relation to the venter is typical of males (Fig. 7). Furthermore, Imamura states the “feature and shape of dorsal shields are all similar to those of the female” (Imamura 1976). Again, testudacarine male and female dorso-glandularia-4 are positioned differently with respect to the muscle scars. While his word choice of “similar” suggests this difference could exist, without a more elaborate description or a drawing it is impossible to tell (Imamura 1976, 1980). In short, it is possible that this is an atypically small female, or a teneral female that has not undergone secondary growth and sclerotization. Imamura (1976) continues to confuse sexual dimorphism when he states: “the female of *okadai* n. sp. is also clearly distinguished from… *japonicus*… by the feature of the venter.” Although this is true, it is because one is female and the other male. This casts suspicion on *T. okadai*. Imamura (1976) seems to be suggesting they are separate species based on his confusions about sexual differences. *Testudacarus okadai* could be synonymous with *T. japonicus* and this issue should be further explored. Wiles (1997a) offers a key to Asian species, but the characters he used to differentiate species are also differences between sexes and therefore are not useful.

*Testudacarus binodipalpis* Guo & Jin, 2005
http://zoobank.org/0AF046B5-9FA7-4DA2-9C08-7051076DA7AC


The types were not examined for this publication; contact with the authors was attempted but unsuccessful and the the types were not examined.

Type deposition. Institute of Entomology, Guizhou University.
**Diagnosis.** These mites can differ from all other Testudacarinae by distribution (China) and from *T. tripeltatus* by their small size (dorsal length <700 µm). More research and updated descriptions are needed for a better diagnosis.

**Distribution.** Mt. Fanjing (Guo and Jin 2005) and Fujian, China (Jin et al. 2010).

**Remarks.** *Testudacarus binodipalpis* was described from one female and one “male.” The described “male” is almost certainly a female as it exhibits all female sexual characters and no ejaculatory complex is noted in the description. However, these two females differ in some noteworthy respects. From illustrations it appears that the smaller female seems to have undergone tertiary sclerotization, while the larger female seems to have only undergone primary and secondary sclerotization. The size and positioning of lateral platelets are also quite different in each specimen. For these reasons the specimens should be re-examined as they might represent two species diagnosable by size. Guo and Jin (2005) state that *T. binodipalpis* can be separated from other *Testudacarus* by “the possession of 2 tubercles on the ventral surface of the” pedipalp tibia and the genu and femur “both with a feathered seta on the ventral surface.” These pedipalp characters do not work as they are plesiomorphic for all *Testudacarus* (Fig. 5). Guo and Jin (2005) also state that the “dorsal and ventral apodeme both [have] a round terminal tip; [coxae-IV] with a triangular base.” These additional characters are unhelpful in separating any testudacarines.

**Key to world species of Testudacarinae**

1. Pedipalp four-segmented, anterior tip of coxa-I with projection; California................................................................. *D. oribatoides*

   – Pedipalp five-segmented, anterior tip of coxa-I without projection ..........2

2. Body elongate to rectangular............................................. (*T. elongatus* complex)........3

   – Body oval.................................................................................................5

3. Lateral platelets robust; lateral-platelet-4 large and in direct or near direct contact with lateral-platelet-2.......................................................4

   – Lateral-platelet-4 small, gap present between it and lateral-platelet-2; widespread along west coast of N. America............................................... *T. oblongatus*

4. Distribution restricted to the Olympic Mountains.............. *T. rectangulatus*

   – Distribution within and east of the Cascade Mountains .......... *T. elongatus*

5. Body large (>700 µm female and >650 male dorsal length; if smaller, found in Asia), dull coloration common; within and west of the Rocky Mountains or Asia ............ (Asian species and *T. americanus* complex except *T. rollerae*)........6

   – Body small (<700 µm female and <650 male dorsal length), bright coloration (orange, red, violet, blue) common; present throughout North America ..........(T. minimus complex, T. hitchensi complex, T. rollerae)..................12

6. Gnathosomal bay “covered” (short dorsal gnathosomal bay length), gnathosoma elongate with long rostrum that extends below ventral surface of gnathosoma................................................................. *T. hyporhynchus*

   – Without these characters........................................................................7
Body very large (>900 µm dorsal length, female) with small, square (unusual for complex) anterio-medial platelet (male unknown).............. *T. kirkwoodae*

- Body not this large (<900 µm dorsal length, female and male) often with wide, rectangular or trapezoidal anterio-medial platelet ..........................8

8 Anterior-medial platelet compact and pentagonal, suture lines between second and third coxae absent; India, Java, Bhutan ....................... *T. tripeltatus*

- Anterior-medial platelet wider, more trapezoidal in shape, suture lines between second and third coxae present, but incomplete ..................9

9 Body small (<650 µm dorsal length female and <450 µm dorsal length male); Japan ....................................................... *T. japonica* or *T. okadai*

- Body larger (>700 µm dorsal length female and >500 µm dorsal length male) .................................................................10

10 Body <770 µm dorsal length female and <660 µm dorsal length male, dg-4 in line with middle of dorsal muscle scars; China.................... *T. binodipalpis*

- Body >780 µm dorsal length female and >670 µm dorsal length male, dg-4 anterior to dorsal muscle scars..................................................11

11 Body elliptical, colorless to peach, small cheliceral fang (<33 µm)............

- Body rounded, grey to colorless, large cheliceral fang (>40 µm) ....... *T. smithi*

12 Anterio-medial platelet wide (>140 µm) and more than or nearly twice as wide as long............................................................ *T. rollerae*

- Anterio-medial platelet unmodified (<140 µm) and less than twice as wide as long ........................................(T. minimus and T. hithensi complex)........13

13 Anterio-medial and anterio-lateral platelets with consistent coloration (either colorless or colored across).................................(T. minimus complex)........14

- Anterio-lateral platelets with coloration and anterio-medial platelet colorless......18

14 Body in entirety conspicuously violet; currently known only from Arkansas......

- Body orange, red, pink, blue, or violet but not covering the majority of body .................................................................15

15 Distribution east of the Great Plains, color violet to blue...........*T. vulgaris*

- Distribution within and west of the Great Plains..................................................16

16 Distribution in Washington or northern Oregon, orange to red ............

- Distribution in and west of Great Plains excluding Washington and northern Oregon .................................................................17

17 Violet to blue, females and males smaller than 600 and 500 µm, respectively...

- Collected outside of California, orange to red to clear, females and males larger than 600 and 500 µm, respectively...........*T. minimus* (most likely)

- Collected in California and orange to clear ...........................................

- *T. minimus, vulgaris, or deceptivus* (no morphological characters for reliable identification available within California)
Dorsal plate with large medial pores surrounded by distal ring of smaller pores (all pores uniform in other species), area posterior to coxal plate “bleached” in males ................................................................. \textit{T. hitcensi}

– Dorsal plate with uniform pores, males without “bleached” area..................19

Anterio-lateral platelets with violet to blue coloration covering most of platelet ................................................................. \textit{T. harrisi}

– Anterio-lateral platelets with coloration restricted to the posterior half of platelet......................................................................................................20

Females and males with dorsal lengths less than 575 and 450 µm, respectively .......................................................................................................................... \textit{T. dennetti}

– Females and males with dorsal lengths greater than 600 and 475 µm, respectively................................................................. \textit{T. dawkinsi}

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References


Hawkins CP (2009) Revised invertebrate RIVPACS model and O/E index for assessing the biological condition of Colorado streams. Prepared by Western Center for Monitoring
and Assessment of Freshwater Ecosystems, Department of Watershed Sciences, Utah State University for Colorado Department of Public Health and Environment, Water Quality Control Division–Monitoring Unit, 42 pp.


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Lundblad O (1941) Eine Übersicht des Hydrachnellensystems und der bis jetzt bekannten Verbreitung der Gattungen dieser Gruppe. Zoologiska Bidrag från Uppsala 20: 359–379. [In German]


Richards AB, Rogers DC (2011) List of Freshwater Macroinvertebrate Taxa from California and Adjacent States Including Standard Taxonomic Effort Levels. Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT), 266 pp.


DNA barcoding reveals polymorphism in the pygmy grasshopper *Tetrix bolivari* (Orthoptera, Tettigidae)

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Abstract

Many pygmy grasshopper species exhibit colour-marking polymorphism. However, this polymorphism in some species, such as *Tetrix bolivari*, is almost unknown. The aim of this work is to identify using DNA barcoding the colour-marking polymorphic morphs of this pygmy grasshopper species collected from both grass and sand microhabitats. Analysis by NJ clustering and pairwise distances indicated that all specimens collected showing colour-marking polymorphism are species of *T. bolivari*. Haplotype network construction showed ten different haplotypes from a total of 57 *T. bolivari* individuals with H1(82.5%) being the most common type and it also displayed low divergence within *T. bolivari* population. The haplotype analyses were consistent with the NJ clustering. Our field census showed the frequency of *T. bolivari* morphs differed significantly, with the rank order of morphs (from high to low) type A₁, type B₁, type A₂, type A₃, type A₄, type A₅, type B₂, type B₃, and type B₄. The most common type A morphs were without contrasting markings, while the rarer type B morphs have contrasting white markings. We suggest that type B morphs have greater camouflage effects against natural backgrounds such as grass or sand than type A morphs. Both our field census and haplotype analysis revealed that type A has higher frequency and more haplotypes than type B.

Keywords

Crypsis, DNA barcoding, frequency, polymorphism, *Tetrix bolivari*
Introduction

Pygmy grasshoppers are typical examples of polymorphic species (Holst 1986, Ichikawa et al. 2006). Different species show colour-marking polymorphism. Moreover, some species are highly polymorphic in colour and markings even within a single population (Forsman 2000). Such polymorphism has adaptive significance which can provide camouflage for the species against their natural backgrounds (crypsis), such as grass or sand (Ruxton et al. 2004, Stevens and Merilaita 2009, Cott 1940, Thayer 1909). The degree of camouflage differed among the colour-marking polymorphic morphs. Usually morphs with contrasting markings have greater camouflage against their natural backgrounds than those without them. However, the degree of camouflage of the morphs was not consistent with the morph frequency. It was reported the frequency of morphs with various types of markings differed significantly between the grass and sand microhabitats. Overall, morphs with contrasting markings were rarer in both microhabitats, although they were more cryptic (Kaori et al. 2010). The more cryptic morphs are not common in the microhabitats. Furthermore, for certain morphs with contrasting marking, such as longitudinal morphs of *Tetrix japonica*, they tended to be more common in the sand microhabitat where they were more conspicuous compared to the grass background where the markings provided a stronger camouflage effect for them. Therefore, the morph frequency cannot reflect the degree of crypsis.

Many pygmy grasshopper species exhibit discontinuous variation in colour and pattern of the pronotum, such as *Tetrix japonica* (Kaori et al. 2010), *Tetrix undulate* (Ahnesjo and Forsman 2003) and *Tetrix subulata* (Forsman 1997, 2000, 2001) and there is a strong tendency for the general patterns to be repeated in different genera and species (Nabours 1929, Fisher 1939). However, there are no publications on polymorphism in pygmy grasshoppers in China.

The Tetrigidae is an ancient group of Orthoptera with relatively uniform body structure. Most Tetrigidae are small, inconspicuous orthopterans about one cm long. They are terricolous and inhabit humid habitats, and some species are semi-aquatic (Podgornaya 1983, Paranjape et al. 1987).

In China, the Family Tetrigidae contains 15 genera, including the large genus *Tetrix*, which currently has 88 species (Deng et al. 2008). Generally, this Family of grasshoppers is among the least-studied groups of Orthoptera and most publications mainly focused on descriptions of new species and morphological taxonomy in China. Their ecology and biology are almost unknown.

*Tetrix bolivari* is one of the least studied species of China Tetrigidae but with wide distribution. Few published reports provide molecular data about its phylogeny (Fang et al. 2010, Chen and Jiang 2004, Jiang et al. 2002), and data on the activity, feeding biology and vibratory communication can be found in one study (Petr et al. 2011). Polymorphism within *T. bolivari* adults has not been reported.

In this paper, we collected pygmy grasshoppers from both grass and sand microhabitats with large variation in body colouration and markings. To examine whether
they are the same species with various colour-marking morphs or they are the different species, we conducted identification experiments using the protein-coding cytochrome c oxidase subunit I (COI) region as a DNA barcode. In addition, we conducted a field census of the morphs in the microhabitats (sand and grass) to confirm the grasshopper morph frequency.

**Materials and methods**

**Sampling**

Adult pygmy grasshoppers were collected from both grass and sand microhabitats in Mianyang, Sichuan Province, China in July to August, 2013. All morphs are characterized by both a long pronotum that extends beyond the apex of the abdomen and highly reduced forewings. They are small (males, 11.8–16.0 mm; females, 13.5–17.0 mm) and exhibit extraordinary variation in the colour and markings of the pronotum from black, through yellowish-brown to light grey or white, with some individuals being monochrome and others having spots, markings or distinct patterns on the pronotum (and also on the hind legs) such as a narrow light yellowish longitudinal stripe on the mid-line of the upper surface of the pronotum or whitish and blackish markings on the dorsal surface of the pronotum.

Fifty-seven specimens of different colour-marking morphs were preserved in 100% ethanol and stored at −4 °C for identification experiments.

**Identification experiments**

DNA extraction: DNA from the tissues of the grasshoppers was extracted from the hind leg using a routine phenol/chloroform method (Zhou et al. 2007).

PCR amplification and sequencing: The DNA was amplified using polymerase chain reaction (PCR) in an ABI thermocycler. The following primers were used for amplification of the COI gene: 5’-TYTCAACAAYCAYAARGATATTGG-3’ and 5’-TAACTTCWGGRTGWCCAAARAA TCA-3’. PCR reaction was carried out in a total volume of 15 ul containing 1 ul DNA template, 7.5 ul Mix (2×Taq DNA Polymerase, 2×PCR Buffer, 2×dNTP), 1 ul of each primer and 4.5 ul PCR-grade RNase-free water. Thermo-cycling conditions were as follows: one initial cycle of 4 min at 94 °C followed by 35 cycles of 94 °C for 15 s, 46 °C for 20 s, 70 °C for 90 s, with final step of 72 °C for 7 min. The PCR products were visualized on 1% agarose gels and then sequenced with both the forward and reverse primers by Shanghai SAN-GON after separation and purification.

Data analysis: The 57 sequences were aligned using CLUSTALX and the standard 658bp was kept for the following analysis (GenBank Accession nos KU570134-KU570190). The morphological identification for one individual (No. 57) suggested it
was *Tetrix bolivari* (Tetrigidae, Tetriginiae, *Tetrix*). The 56 remaining individuals plus the individual *T. bolivari* were analyzed together in NJ analysis by MEGA version 5.0, together with another 4 *Tetrix* species, 1 *Alulatettix yunnanensis* and *Teleogryllus emma* as outgroup. The Kimura 2-parameter (K2P) model of base substitution was used to calculate pairwise genetic distance in MEGA 5 software. Species discrimination in DNA barcoding studies also depends on establishment of threshold interspecies nucleotide divergence. In this study, nucleotide divergence of 3% was considered as a threshold between species as observed in Orthopterans (Mao 2011).

The haplotype network based on 658 base pairs of COI sequences was constructed using the median-joining algorithm (Bandelt et al. 1999) implemented in NETWORK 4.1 (Forster et al. 2000).

**Field survey of the frequency of grasshopper morphs**

Grasshoppers were collected using random sweeps with an insect net in both microhabitats and we counted the different colour-marking morphs in the field. Only adults were used in this research.

**Results**

**Identification by DNA barcoding**

Our NJ analysis showed that the 56 individuals with different colour-marking morphs clustered with *T. bolivari* into one clade with bootstrap value of 100% (Fig. 1). The pairwise distances indicated the nucleotide divergence varied from 0.0% to 0.6% and the overall mean distance was 0.1%, which was far less than the threshold of 3% used for species discrimination (Suppl. material 1). According to the above analysis, we inferred that all pygmy grasshoppers with different morph types in this research are all species of *T. bolivari*. Meanwhile, the NJ analysis showed that *T. bolivari, T. qinlingensis, Alulatettix yunnanensis* and *T. japonica* have close relationship with a bootstrap value of 100%. However, another two species *T. ornata* and *T. brunnerii* were separated from the other species by interspecies nucleotide divergence ranging from 16.1% to 18%.

**Haplotype network construction**

The haplotype analyses based on the 62 sequences were consistent with the NJ clustering (Fig. 2A). Ten different haplotypes were found in *T. bolivari* population (Fig. 2). The most frequent haplotype was H1, occurring in 82.5% of individuals sampled (Table 1), indicating a low degree of variation in this population. This type was followed
DNA barcoding reveals polymorphism in the pygmy grasshopper *Tetrix bolivari*... 

**Figure 1.** NJ clustering analysis of 62 sequences of pygmy grasshoppers using MEGA5 and K2P model. *Teleogryllus emma* is used as an outgroup. The clade 1-57 represents samples from this study.

**Figure 2.** A Haplotype network from 63 sequences, including 57 *Tetrix bolivari* individuals, 4 *Tetrix* species, 1 *Alulatettix yunnanensis* and 1 outgroup (*Teleogryllus emma*). B Haplotype network of 57 *Tetrix bolivari* individuals combined with the morph types. Circle size is proportional to haplotype frequency. Lines drawn between haplotypes represent mutation events identified by the numbers corresponding to the positions at which the mutations were observed. Red points represent hypothetical haplotypes (median vector). Colours in B represent morph types. Yellow areas represent type A and black areas represent type B.

by H4 with much lower frequency (3.5%) and the remaining haplotypes occurred at a frequency of 1.8% (Table 1). Haplotypes H3, H4, H5, H6, H8 and H9 were each linked to H1 by a single nucleotide substitution at positions 3, 394, 343, 355, 28 and 235, respectively (Fig. 2A). The haplotype H7 was the most distant haplotype. It was separated from the haplotype H1 by 3 mutational events (Fig. 2).
Table 1. Frequency of the ten different haplotypes based on the 658 bp COI region in 57 Tetrix bolivari individuals used in this study.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Sequence code</th>
<th>Frequency (%)</th>
<th>Morph type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>H1</td>
<td>1 3 4 5 6 7 8 9 11 12 13 14 15 16 19 21 22 23 24 25 26 28 29 30 31 32 34 35 36 37 38 39 40 41 42 44 45 46 47 48 49 50 51 53 54 56 57</td>
<td>47 (82.5)</td>
<td>40</td>
</tr>
<tr>
<td>H2</td>
<td>33</td>
<td>1 (1.8)</td>
<td>1</td>
</tr>
<tr>
<td>H3</td>
<td>43</td>
<td>1 (1.8)</td>
<td>1</td>
</tr>
<tr>
<td>H4</td>
<td>2 18</td>
<td>2 (3.5)</td>
<td>2</td>
</tr>
<tr>
<td>H5</td>
<td>55</td>
<td>1 (1.8)</td>
<td>1</td>
</tr>
<tr>
<td>H6</td>
<td>10</td>
<td>1 (1.8)</td>
<td>1</td>
</tr>
<tr>
<td>H7</td>
<td>20</td>
<td>1 (1.8)</td>
<td>1</td>
</tr>
<tr>
<td>H8</td>
<td>17</td>
<td>1 (1.8)</td>
<td>1</td>
</tr>
<tr>
<td>H9</td>
<td>27</td>
<td>1 (1.8)</td>
<td>1</td>
</tr>
<tr>
<td>H10</td>
<td>52</td>
<td>1 (1.8)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td></td>
<td>49</td>
</tr>
</tbody>
</table>

Frequency of different colour-marking morphs in the grass and sand microhabitats

A total of 343 pygmy grasshoppers (T. bolivari) were collected from both microhabitats and we categorized these morphs into 2 types (type A and type B) and 11 subtypes (type A_{1-7}; and type B_{1-4}) based on the colour and markings on the pronotum (Fig. 3). Type A generally has dark colours, such as black, brown and grey, without contrasting markings. While type B had white on the pronotum, mixed with the black markings. The number of each subtype is shown in Table 2. From this table, we can see that type A was dominant in the habitat with type A_{1} more common than other subtypes (type A, 79.3%; type A_{1}, 29.7%), whereas type B was rare, especially type B_{4} which has obvious contrasting markings (type B, 20.7%; type B_{4}, 0.9%).

In this study, 57 T. bolivari individuals were used in NJ clustering and haplotype analysis, including 49 type A morphs and 8 type B morphs (Table 1). These type A morphs have nine different haplotypes and the 8 type B morphs have two haplotypes (Fig. 2B). Both morph types have haplotype H1 and it is the most prevalent type in both type A and type B.

Discussion and conclusion

The aim of this study was to identify morphs of T. bolivari using DNA barcoding and examine polymorphism and morph frequency in T. bolivari. Both the NJ clustering analysis and pairwise distances indicated that all specimens in this experiment are species of T. bolivari, which exhibits polymorphism in colour-marking morphs. Furthermore, we found the non-marked morph, spotted morph, and horizontal morph in T.
DNA barcoding reveals polymorphism in the pygmy grasshopper *Tetrix bolivari*.

**Table 2.** Morph types of pygmy grasshoppers (*Tetrix bolivari*) classified by the colour and markings on the pronotum.

<table>
<thead>
<tr>
<th>Type</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₈</td>
<td>102</td>
<td>29.7</td>
</tr>
<tr>
<td>A₉</td>
<td>34</td>
<td>9.9</td>
</tr>
<tr>
<td>A₁₀</td>
<td>31</td>
<td>9.0</td>
</tr>
<tr>
<td>A₁₁</td>
<td>31</td>
<td>9.0</td>
</tr>
<tr>
<td>A₁₂</td>
<td>28</td>
<td>8.2</td>
</tr>
<tr>
<td>A₁₃</td>
<td>25</td>
<td>7.3</td>
</tr>
<tr>
<td>A₁₄</td>
<td>21</td>
<td>6.1</td>
</tr>
<tr>
<td>A₁₅</td>
<td>272</td>
<td>79.3</td>
</tr>
<tr>
<td>B₁</td>
<td>43</td>
<td>12.5</td>
</tr>
<tr>
<td>B₂</td>
<td>15</td>
<td>4.4</td>
</tr>
<tr>
<td>B₃</td>
<td>10</td>
<td>2.9</td>
</tr>
<tr>
<td>B₄</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td>B₁₋₄</td>
<td>71</td>
<td>20.7</td>
</tr>
</tbody>
</table>

**Figure 3.** Morphs of pygmy grasshoppers (*Tetrix bolivari*) classified by type of colour and markings on the pronotum. A₁₋₇ belongs to type A; B₁₋₄ belongs to type B.

*japonica* also appeared in *T. bolivari*. It was reported *T. bolivari* is commonly found with *T. subulata* (Adamovic 1969, Sardet 2007). Therefore, we suspect these two species presumably share some morph types, although we didn’t find any *T. subulata* in our collection.
Our NJ analysis showed that *Alulatettix* was closely related to the three *Tetrix* species. Previous molecular studies (Fang et al. 2010) correspond with results of our research. From the morphological observations, *Alulatettix* and *Tetrix* belong to the subfamily Tettiginae, and both of these genera are similar in the head not projecting above upper level of pronotum and the posterior margin of lateral lobes in lateral view with two concavities, but they differ in the shape of pronotum and degree of development of tegmina and hind wings. However, another two *Tetrix* species (*T. ornata* and *T. brunnerii*) were less close to the other species and showed higher interspecies nucleotide divergence, from 16.1% to 18%. Here, sequences of *T. ornata* and *T. brunnerii* downloaded from GenBank were submitted by the Biodiversity Institute of Ontario, Canada, a place far from China, while the other 2 *Tetrix* species (*T. japonica* and *T. qinlingensis*) and *A. yunnanensis* were contributed by Nanjing Normal University, China and *T. bolivari* was from our lab. So, we inferred that the higher nucleotide divergence between *T. ornata*, *T. brunnerii* and other pygmy grasshopper species likely reflects geographical distribution differences. In addition, the molecular phylogeny also revealed that the genus *Tetrix* is not monophyletic (Fang et al. 2010, Chen and Jiang 2004).

Furthermore, a total of ten different haplotypes were detected in this single colour-marking polymorphic population with H1 (82.5%) being the most common type. The haplotype network displays a shallow divergence in this *T. bolivari* population with a maximum of 4 base changes between the most divergent haplotypes. The haplotype analysis combined with the morph types showed type A has more haplotypes than type B and both of them have the prevalent haplotype H1.

Our field census of the polymorphism in the microhabitats (sand and grass) demonstrated that the different morphs of *T. bolivari* were not equivalent in the frequency, with the rank order of morphs (from high to low) being type A1, type B1, type A2, type A3, type A4, type A5, type A6, type A7, type B2, type B3, type B4. Generally, type A was more common than type B. Earlier work on *T. japonica* has revealed the more common morphs, usually without contrasting markings, are not more cryptic in either grass or sand microhabitat. In contrast, the more cryptic morphs which have contrasting markings were rarer in each microhabitat (Kaori et al. 2010). Our field survey also showed that the more common type A morphs usually exhibited non-marked and mono-coloured basal colouration or any number of spots on mono-coloured basal colouration, while the rarer type B morphs had contrasting white markings. So, we infer that type B morphs have a much greater degree of crypsis against the natural backgrounds, such as grass or sand than type A morphs. There is no positive association between morph frequency and the degree of crypsis, which can be explained by the differential crypsis hypothesis (Forsman 1998).

Acknowledgments

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References


Nabours RK (1929) The genetics of the Tettigidae (Grouse Locusts). Bibliographia Genetica 5: 27–104.


Podgornaya LI (1983) Pryamokrylye nasyekomye semeystva Tettigidae (Orthoptera) fauny SSSR. Trudy Zoologicheskogo Instituta AN SSSR, 112.


Sardet E (2007) *Tetrix bolivari* Saulcy in Azam, 1901, espèce mythique ou cryptique? (Caelifera, Tettigioidea, Tettigidae) (Is *Tetrix bolivari* Saulcy in Azam, 1901, mystic or cryptic species? (Caelifera, Tettigioidea, Tettigidae)). Matériaux Orthoptériques et Entomocénétiques 12: 45–54. [In French]


**Supplementary material I**

**Pairwise genetic distance (K2P) based on COI sequence using MEGA 5**

Authors: Ling Zhao, Li-Liang Lin, Zhe-Min Zheng

Data type: specimens data

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Aspilota isfahanensis, a new species of the genus Aspilota Foerster, 1863 from Iran (Hymenoptera, Braconidae, Alysiinae)

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http://zoobank.org/1BDE4B9D-2FEE-4E58-8B31-F17188EE9C9A

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Abstract
A new species of Aspilota without mesoscutal pit, A. isfahanensis Peris-Felipo, sp. n., is described and illustrated from Iran. The new species is compared with its three morphologically most similar species, A. compressiventris Stelfox & Grahan, 1951, A. makita Papp, 2008 and A. spiracula Munk & Peris-Felipo, 2013, is provided. A key to the western Asian species of Aspilota is provided.

Keywords
Alysiinae, Aspilota, new species, identification key, Palaearctic, Iran

Introduction
The complex of genera that are closely related to Aspilota is the most taxonomically complicated group within the braconid Alysiinae, mainly because of their small body size and their reduced number of available diagnostic characters (Belokobylskij 2005).
The genus *Aspilota* Foerster, 1863, is well defined by the presence of the paraclypeal fovea connecting with inner margin of eye and of the vein cuqu1 (2-SR) of the fore wing (van Achterberg 1988; Peris-Felipo and Belokobylskij 2016).

Information about *Aspilota* species from Western Asia is scarce, and only two species have been previously recorded, both from Iran (Yu et al. 2012; Gadallah et al. 2015). In this work, an additional new species of *Aspilota* from Iran (Isfahan Province) is described. The new species is compared with its three morphologically similar Palaearctic species, *A. compressiventris* Stelfox & Grahan, 1951, *A. makita* Papp, 2008 and *A. spiracula* Munk & Peris-Felipo, 2013, is provided. Finally, a key to the three western Asian species of *Aspilota* is given.

**Material and methods**

For the terminology of the morphological features, sculpture and measurements, see Peris-Felipo et al. (2014); for wing venation nomenclature, see Peris-Felipo et al. (2014) and in parenthesis van Achterberg (1993). The keys by Fischer (1976, 1978), Belokobylskij and Tobias (2007) and Papp (2008) were used for the identification of the new *Aspilota* species. The material was imaged using Digital Microscope Keyence® VHX-2000 and Adobe Photoshop® imaging system. The types of the new species are deposited in the collections of the Naturhistorisches Museum (Vienna, Austria; NHMW) and Zoological Institute of the Russian Academy of Sciences (St Petersburg, Russia; ZISP).

**Taxonomy**

Order *Hymenoptera* L., 1758  
Family *Braconidae* Nees, 1811  
Subfamily *Alysiinae* Leach, 1815  
Genus *Aspilota* Foerster, 1863

*Aspilota isfahanensis* Peris-Felipo, sp. n.  
http://zoobank.org/A4282F26-0353-4FFC-B8B5-2A13784E3C2B  
Figs 1, 2

**Type material.** Holotype: female, Iran, Isfahan, 6.x.2012, sweep net on *Chenopodium* sp. (E. Nader leg.) (NHMW). Paratype: 1 ♀, same data as for holotype (ZISP).

**Description.** Female (holotype).

**Head.** In dorsal view, 1.9 times as wide as its median long, 1.4 times as wide as mesoscutum, smooth, with temple rounded behind eyes (Fig. 1F). Eye in lateral view 1.4 times as high as wide and 1.7 times as wide as temple medially (Figs 1B, 2A). POL 1.6 times OD; OOL 3.0 times OD (Fig. 1F). Face 1.9 times as wide as high; inner
Aspilota isfahanensis, a new species of the genus Aspilota...

Margins of eyes subparallel (Fig. 1E). Clypeus 2.5 times as wide as high, slightly curved ventrally (Fig. 1E). Paraclypeal fovea reaching inner margin of eye (Fig. 1E). Mandible 3-dentate, weakly widened towards apex, 1.3 times as long as its maximum width. Upper tooth distinctly shorter than lower tooth, very small and rounded; middle tooth rather long and narrow, longer than lower tooth, pointed apically; lower tooth widest, rounded, distinctly moving downwards (Fig. 1C). Antennae 17-segmented, 0.8 times as long as body. Scape 2.4 times longer than pedicel. First flagellar segment 3.3 times as...
long as its apical width, 1.3 times as long as second segment. Second flagellar segment
2.2 times as long as its maximum width; third to twelfth segments about 1.8 times as
long as their maximum width, 13th and 14th segments 2.0 times, and 15th (apical)
segment 2.5 times as long as their wide accordingly (Fig. 1D).

Mesosoma. In lateral view, 1.2 times as long as high (Fig. 2A). Mesoscutum (dorsal
view) 0.8 times as long as its maximum width, smooth, with two lines of sparse setae
along tracks of notauli (Fig. 2B). Notauli mainly absent on horizontal surface of mesos-
cutum (Fig. 2B). Mesoscutal pit absent (Fig. 2B). Prescutellar depression smooth, with-
out lateral carinae (Fig. 2B). Precoxal sulcus present, crenulate, not reaching anterior
and posterior margins of mesopleuron (Fig. 2A). Posterior mesopleural furrow crenu-
late in upper half, smooth in lower half (Fig. 2A). Propodeum with pentagonal areola
delineated by distinct carinae (Fig. 2C). Propodeal spiracles relatively small (Fig. 2C).

Wings (Fig. 2F). Length of fore wing 2.7 times as long as its maximum width. Radial (marginal) cell ending at apex of wing, 4.0 times as long as its maximum width. Vein r2 (3-SR) 2.3 times as long as vein cuqu1 (2-SR); vein r3 (SR1) 2.5 times as long
as vein r2 (3-SR). Nervulus (cu-a) distinctly postfurcal. Brachial (subdiscal) cell closed
distally, 3.0 times as long as its maximum width. Hind wing 6.5 times as long as its
maximum width.

Legs (Fig. 2E). Hind femur claviform, 4.0 times as long as its maximum width. Hind tibia weakly widened towards apex, 9.7 times as long as its maximum subapical
width, 1.5 times as long as its hind tarsus. First segment of hind tarsus twice as long
as second segment.

Metasoma. First tergite long, slightly widened towards apex, 2.6 times as long as
its apical width, finely rugose-striate in apical half (Fig. 2D). Ovipositor 1.2 times as
long as first tergite, 0.4 times as long as metasoma, 0.9 times as long as hind femur, 0.2
times as long as fore wing (Fig. 2E).

Colour. Body, antenna, and pterostigma dark brown. Mandibles and legs yellowish
brown. Wings hyaline. Length. Body 1.8 mm; fore wing 2.0 mm; hind wing 1.8 mm.


Male. Unknown.

Etymology. Named after Isfahan, the type locality of new species.

Comparative diagnosis. This new species is similar to A. compressiventris Stel-
fox & Grahan, 1951 (Austria, Hungary, Russia, and U.K), A. makita Papp, 2008
(Hungary and Romania) and A. spiracula Munk & Peris-Felipo, 2013 (Denmark).
All these species have the propodeum with a pentagonal areola delineated by a distinct
carinae. However, A. isfahanensis sp. n. differs from A. compressiventris in having the
mandible 1.3 times as long as its maximum width (1.7 times in A. compressiventris),
the first flagellar segment 3.3 times as long as its maximum width (4.0 times in A.
compressiventris), the hind femur 4.0 times as long as its maximum width (4.5 times
in A. compressiventris), the first metasomal tergite 2.6 times as long as its apical width
(3.0–4.0 times in A. compressiventris), the face 1.9 times as long as high (1.5 times in
A. compressiventris), and the head in dorsal view 1.9 times as long as long (1.5 times in
A. compressiventris). The new species differs from A. makita in having the mandible 1.3
Aspilota isfahanensis, a new species of the genus Aspilota... times as long as its maximum width (1.7 times in A. makita), a hind femur 4.0 times as long as its maximum apical (3.2 times in A. makita), the first metasomal tergite 2.6 times as long as its apical width (2.0 times in A. makita), a propodeum with the areola distinctly delineated by carinae (areola less distinctly delineated in A. makita), the first flagellar segment 3.3 times as long as its maximum width (4.0 times in A. makita), and the upper tooth rounded (pointed in A. makita). Finally, A. isfahanensis sp. n. differs from A. spiracula in having the mandible 1.3 times as long as its maximum width (1.5
times in *A. spiracula*), the eye in lateral view 1.7 times as wide as the temple medially (nearly as long in *A. spiracula*), the first flagellar segment 3.3 times as long as its maximum width (2.5 times in *A. spiracula*), middle flagellar segments 1.8–2.2 times as long as their maximum widths (1.0–1.1 times *A. spiracula*), the first metasomal tergite 2.6 times as long as its apical width (2.3 times in *A. spiracula*), and a long metasoma (short in *A. spiracula*).

**Key to the western Asian species of *Aspilota***

1. Eye in lateral view 0.5–0.8 times as wide as temple medially. First metasomal tergite about 2.0 times as long as its apical width. Hind femur 4.5–5.0 times as long as its maximum width. .................................................. 2

  - Eye in lateral view 1.7 times as wide as temple medially (Fig. 1B, 2A). First metasomal tergite 2.6 times as long as its apical width (Fig. 2D). Hind femur 4.0 times as long as its maximum width (Fig. 2E). Body length 1.8 mm. Iran................................. *A. isfahanensis* Peris-Felipo, sp. n.

2. Eye in lateral view 0.5 times as wide as temple medially. First flagellar segment 4.0 times as long as its maximum width; middle segments 1.5 times as long as their maximum width. Hind femur 5.0 times as long as its maximum width. Vein r2 (3-SR) 2.0 times as long as vein cuqu1 (2-SR). Body length 1.8 mm. Iran ................. *A. alfalfae* Fischer, Lashkari Bod, Rakhshani & Talebi, 2011

  - Eye in lateral view 0.8 times as wide as temple medially. First flagellar segment 3.5 times as long as its maximum width; middle segments 1.8 times as long as their maximum width. Hind femur 4.5 times as long as its maximum width. Vein r2 (3-SR) 2.5 times as long as vein cuqu1 (2-SR). Body length 1.8–2.2 mm. Austria, Greece, Hungary, Iran....... *A. delicata* Fischer, 1973

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Aspilota isfahanensis, a new species of the genus Aspilota...

References


Four new species of *Trigonopterus* Fauvel from the island of New Britain (Coleoptera, Curculionidae)

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Abstract

The hyperdiverse genus *Trigonopterus* has its center of diversity in Melanesia, but only a single species is recorded from the Bismarck Archipelago to date. Here we describe four new species from the island of New Britain: *T. chewbacca* sp. n., *T. obsidianus* sp. n., *T. puncticollis* sp. n. and *T. silaliensis* sp. n. We provide cytochrome oxidase subunit I (cox1) sequences of the new species and a key to all five species known from the Bismarck Archipelago.

Keywords

Bismarck Archipelago, cox1, Cryptorhynchinae, DNA barcoding, endemism, hyperdiverse, morphology, New Guinea, Nakanai Range, weevils

Introduction

*Trigonopterus* Fauvel is a genus of flightless weevils of the subfamily Cryptorhynchinae (Alonso-Zarazaga and Lyal 1999). It is distributed in Southeast Asia (Riedel et al. 2014), Australia (Riedel and Tänzler 2016) and Melanesia, with its center of diversity in New Guinea (Riedel et al. 2010; Tänzler et al. 2012, Riedel et al. 2013b). Despite
*Trigonopterus* being recorded from the remote islands of Fiji (Zimmerman 1938b), Samoa (Marshall 1931) and New Caledonia (Heller 1916), only one species has been described from the Bismarck Archipelago to date, i.e. *Trigonopterus pembertoni* (Zimmerman 1938a) from New Ireland. Here we describe four new species from the island of New Britain. Presumably, there are many additional new species to be found on this island. Unfortunately, large expanses of low-elevation forests in New Britain have been converted to oil-palm plantations, highlighting the significance of documenting the insect fauna before the remaining forests are gone.

**Materials and methods**

This study is based on 18 specimens, the result of a ten-day expedition to the area east of Silali Village in the Nakanai Range of West New Britain during November of 2014 by the first two authors. Specimens were collected by beating foliage and by sifting of leaf litter with subsequent extraction of specimens using Winkler eclectors (Besuchet et al. 1987). Holotypes were selected from the ten sequenced specimens; their DNA was non-destructively extracted as described by Riedel et al. (2010). Preparation of genitalia follows the method described by Riedel et al. (2014). Illustrations of habitus and genitalia are of holotypes.

Type depositories are cited using the following codes:

- **ANIC** Australian National Insect Collection, Canberra, Australia
- **SMNK** Staatliches Museum für Naturkunde, Karlsruhe, Germany.
- **ZSM** Zoologische Staatssammlung, München, Germany.
- **UPNG** University of Papua New Guinea, Entomology Collection

DNA sequencing and sequence analysis follows the method described by Riedel et al. (2010) and Tänzler et al. (2012). In the diagnostic descriptions, only the major characters are given, as in the format outlined by Riedel et al. (2013a, b).

Specimens were studied under a Leica MZ16 dissecting microscope and a fluorescent desk lamp for illumination. Measurements were taken using an ocular grid. Body length was measured in dorsal aspect from the elytral apex to the front of the pronotum, and elytral width between the humeri at their greatest extent and across both elytra. Legs were described in an idealized, laterally extended position; there is a dorsal / ventral and an anterior / posterior surface. Habitus illustrations were prepared by photographing the specimens with a DFC450 camera with L.A.S. 4.6.0 software mounted on a Z6 APO (all from Leica Microsystems, Heerbrugg, Switzerland). Photographs of the genitalia were taken under an Axio Imager M2 microscope (Carl Zeiss Microscopy) equipped with 5X or 10X A-Plan lenses and with a JVC KY70 camera (JVC Professional Products); the resulting image stacks were combined using the Helicon Focus 6.2.2 software (Helicon Soft Ltd). For this purpose the genitalia were embedded in glycerol gelatin, as described by Riedel (2005). The genitalia were photographed with their longitudinal axis somewhat raised at the posterior end, to
adequately illustrate the structures of the curved apex. All photographs were enhanced using Adobe Photoshop CS2. Sequence data were submitted to the GenBank, and the accession numbers are provided under each species e.g. as “(GenBank # KU888894)”.

**Taxonomy**

*Trigonopterus* Fauvel, 1862

**Type species.** *Trigonopterus insignis* Fauvel, 1862, by monotypy.


**Descriptions of the species**

*Trigonopterus chewbacca* Van Dam & Riedel, sp. n.

http://zoobank.org/1EA211AA-4D08-4B65-8C3B-9A305B8BD1C9

**Diagnostic description.** Holotype, male (Fig. 1a–c). Length 3.34 mm. Color black; legs and antenna ferruginous. Body subrhomboid; in dorsal aspect with marked constriction between pronotum and elytron; in profile dorsally convex. Rostrum dorsally with rows of erect, clavate scales; with broad median costa bearing three fine ridges; with pair of sublateral furrows; epistome subglabrous, with sparse long setae, posteriorly forming transverse ridge bearing denticles; median denticle largest. Forehead in middle with denticle; laterally with row of long, erect, clavate scales bordering eye. Pronotum with subapical constriction; anteriorly with coarse punctures and sparse clavate scales, laterally angularly projecting; disk subglabrous, with sparse small punctures; basal margin bordered by row of coarse punctures; laterally subglabrous with sparse coarse punctures, posteriorly with large fovea. Elytra subglabrous, striae weakly marked by rows of minute punctures; intervals flat; laterally few punctures deeply impressed; apex subangulate, medially with suture incised, in profile curved ventrad, slightly beak-shaped, punctate-granulate, with sparse recumbent scales. Femora edentate, coarsely punctate, with sparse suberect scales. Metafemur with dorsoposterior edge markedly denticulate; subapically without stridulatory patch. Tibiae dorsally denticulate, with row of erect scales. Abdominal ventrites 1–2 forming common, subglabrous cavity; lateral rim with sparse scales; ventrite 5 with broad, subglabrous impression, subapically coarsely punctate. Penis (Fig. 1b) with apodemes and transfer apparatus in repose reaching into prothorax, > 4 x longer than body of penis; sides of
Figure 1. *Trigonopterus chewbacca* Van Dam & Riedel, sp. n., holotype; a Dorsal habitus b Lateral habitus c Penis.
New Trigonopterus from New Britain

body parallel, apex subangulate, medially with sparse setae; transfer apparatus flagelliform, enveloped by sclerotized sheath; endophallus at base of body with funnel-shaped sclerite; ductus ejaculatorius basally sclerotized, apical portion broken and missing. **Intraspecific variation.** Length 2.78–3.13 mm. Female rostrum with median ridge only basally; in apical third punctate, epistome without distinct transverse ridge.

**Material examined.** Holotype (ANIC): ARC4224 (GenBank # KU888903), Papua New Guinea: West New Britain Prov.: E of Silali Village, Nakana Range, S05°31.233’, E151°03.343’, 800 m, from leaf litter, 21-22-XI-2014. Paratypes (SMNK, UPNG): 2 exx, ARC4222 (GenBank # KU888901), ARC4223 (GenBank # KU888902), same data as holotype.

**Etymology.** This epithet is a noun in apposition and based on the likeable fictional character Chewbacca in George Lucas’ Star Wars movies, portrayed primarily by Peter Mayhew. This species has dense scales on the head and the legs, which reminds the authors of Chewbacca’s dense fur.

**Notes.** Presumably, the species belongs in the *T. basalis*-group of Riedel et al. (2013b).

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**Trigonopterus obsidianus** Van Dam & Riedel, sp. n.
http://zoobank.org/8AE75F70-A3F7-48EC-93C7-C49B2984C215

**Diagnostic description.** Holotype (Fig. 2a–c). Length 2.85 mm. Color black. Body subovate, almost without constriction between pronotum and elytron; in profile evenly convex. Rostrum dorsally sparsely punctate, with pair of sublateral furrows containing rows of scales directed mesad; in front of antennal insertion with shallow lateral constriction. Eye with dorsal margin weakly carinate, bordered by furrow. Forehead with sparse minute punctures. Pronotum with disk subglabrous, with minute punctures; sides above coxa with scattered, coarse punctures; base medially weakly extended towards elytral suture. Elytra subglabrous, including near base and humeri; striae hardly visible. Legs. Femora subglabrous, with minute punctures. Metafemur dorsally with elongate patch of dense silvery scales; posterior surface with pair of longitudinal furrows containing rows of indistinct scales parallel to ventral and dorsal edge; dorsoposterior edge distinct. Mesotibia apically with uncus and separate, much larger premucro. Metatibia in subapical third ventrally with weak swellings, but not denticulate; apically with uncus but no premucro. Abdominal ventrites 1 and 2 medially forming common cavity; ventrite 2 laterally swollen and with suberect scales; posterior edge projecting; ventrite 5 flat, subglabrous, dull, with sparse minute punctures. Penis (Fig. 2b) with sides of body subparallel, weakly concave; apex with median triangular extension, symmetrical; endophallus with short, spiniform transfer apparatus directed ventrad in repose, without distinct sclerites; ductus ejaculatorius without bulbus. **Intraspecific variation.** Length 2.58–2.85 mm. Female mesotibia apically with large uncus and much smaller premucro. Female abdominal ventrites 1 and 2 medially flat.
Figure 2. *Trigonopterus obsidianus* Van Dam & Riedel, sp. n., holotype; a Dorsal habitus  b Lateral habitus  c Penis.
**Material examined.** Holotype (ANIC): ARC4216 (GenBank # KU888896), Papua New Guinea: West New Britain Prov.: E of Silali Village, Nakainai Range, S05°31.233', E151°03.343', 800 m, from foliage, 21-22-XI-2014. Paratypes (SMNK, UPNG): 2 exx, ARC4215 (GenBank # KU888895), ARC4217 (GenBank # KU888897), same data as holotype.

**Etymology.** This epithet is based on the Latin adjective *obsidiansus* and refers to the color of the polished mineral obsidian, which resembles the pronotum and elytra of this species.

**Notes.** This species belongs to the *T. politus*-group of Riedel et al. (2013b) and is similar to *T. politus* (Faust) of the Papuan Peninsula. It can be distinguished by the symmetrical apex of the penis and the less distinct denticles of the male metatibia.

**Trigonopterus puncticollis** Van Dam & Riedel, sp. n.
http://zoobank.org/69951605-F4A2-4EE1-94B0-6A771B7236CA

**Diagnostic description.** Holotype, male (Fig. 3a–c). Length 3.06 mm. Color black, antenna and tarsi ferruginous. Body subovate; with weak constriction between pronotum and elytra; in profile evenly convex. Rostrum in basal half with median and pair of submedian carinae, intervening furrows with rows of yellowish-transparent scales; apical half of rostrum coarsely punctate-rugose, with sparse suberect setae. Forehead coarsely punctate-rugose, punctures with subrecumbent scales pointing backwards. Pronotum coarsely punctate; distance between punctures subequal to their diameter; each puncture containing one inconspicuous seta. Elytra finely punctate, along basal margin with transverse row of deeper and denser punctures; striae impressed as fine lines; marked by rows of small punctures; intervals flat, subglabrous, with few minute punctures; lateral stria behind humerus with dense row of deep punctures. Femora edentate; subapically coarsely punctate, with recumbent scales. Anteroventral ridge of profemur in apical third shortened, forming blunt angulation. Metatibia dorso-posteriorly denticulate; subapically without stridulatory patch. Protibia widening towards apex. Metatibia in apical half with anterior face covered by fringe of long subrecumbent setae. Meso- and metatibia subbasally with dorsal angulation; metatibia with supra-uncal denticle. Abdominal ventrites 1–2 concave with coarse punctures bearing silvery plumose scales; ventrite 5 flat, densely punctate and covered with silvery scales. Penis (Fig. 3b) widening apicad, in apical third with constriction and oblique lateral furrow; apex subangulate, subglabrous; transfer apparatus symmetrical, contained in apical half of body; ductus ejaculatorius without bulbous. **Intraspecific variation.** Length 2.58–2.85 mm. Female mesotibia apically with large uncus and much smaller preuncro. Female abdominal ventrites 1 and 2 medially flat.

**Material examined.** Holotype (ANIC): ARC4220 (GenBank # KU888900), PAPUA NEW GUINEA, West New Britain Prov.: E of Silali Village, Nakainai Range, S05°30.651', E151°02.895', 700 m, from foliage, 21-XI-2014. Paratypes (SMNK, ZSM, UPNG): 9 exx, ARC4218 (EMBL # KU888898), ARC4219 (GenBank # KU888899), ARC4221 (EMBL # XXX), same data as holotype.
Figure 3. *Trigonopterus puncticollis* Van Dam & Riedel, sp. n., holotype; a Dorsal habitus b Lateral habitus c Penis.
**Etymology.** This epithet is a Latin adjective based on a combination of the Latin nouns *punctum* (small hole, dot) and *collum* (neck) and refers to the markedly punctate pronotum.

**Notes.** This species may belong to the *T. oblongus*-group of Riedel et al. (2013b) in a wide sense.

*Trigonopterus silaliensis* Van Dam & Riedel, sp. n.

http://zoobank.org/C6C79A9A-0A51-4252-9603-AF408E46DF26

**Diagnostic description.** **Holotype,** female (Fig. 4a–c). Length 3.02 mm. Color black; elytra with humeri and apical third dark ferruginous; antenna paler ferruginous. Body elongate; in dorsal aspect with marked constriction between pronotum and elytra; in profile dorsally flat. Rostrum basally with median and pair of submedian carinae; in apical half relatively smooth, with submedian row of punctures. Forehead coarsely punctate-rugose, with cream-colored, narrow scales directed backwards. Pronotum with sides convex, without subapical constriction; disk dorsally flat, punctate, at middle subglabrous, punctures sparse; anterolaterally punctures coarse, partly confluent; at middle of basal margin with small patch of dense, cream-colored recumbent scales. Elytra with striae distinct, dorsally with rows of small punctures, laterally with deep punctures; intervals flat, subglabrous; with small, scattered patches of cream-colored, recumbent scales. Legs. Femora edentate; coarsely punctate, with white, recumbent piliform scales. Profemur in basal third posteriorly with callus. Metafemur dorsally with white scales wider and more densely; subapically with stridulatory patch. Abdominal ventrites flat, with fine punctures bearing piliform scales. Genitalia. Fig. 4b.

**Material examined.** Holotype (ANIC): ARC4214 (GenBank # KU888894), PAPUA NEW GUINEA, West New Britain Prov.: E of Silali Village, Nakanai Range, S05°31.233', E151°03.343', 800 m, from foliage, 21-22-XI-2014.

**Etymology.** This epithet is a Latin adjective based on the name of the village near to which the holotype was collected.

**Notes.** This species belongs to the *T. honestus*-group of Riedel et al. (2013b) and superficially resembles *T. paucisquamosus* (Heller) from the Philippines, which differs by the position of scale patches and a denser punctuation.

**Key to the known *Trigonopterus* species of the Bismarck Archipelago of Papua New Guinea**

1 Species found on foliage. Pronotum subapically rounded, without distinct constriction. ..................................................................................................................2

– Species found in the litter layer. Pronotum subapically with distinct constriction and pair of angular projections (Fig. 1a–c) .................................................................................................................................

................................................................. **T. chewbacca** Van Dam & Riedel, sp. n.
Figure 4. *Trigonopterus silaliensis* Van Dam & Riedel, sp. n., holotype; a Dorsal habitus b Lateral habitus c female genitalia.
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2(1) Body larger, pronotum plus elytron ca. 5.63 mm. Elytron black, nude except subapically with elongate patch of white scales. Fig. 5.................................

........................................................................................................T. pembertoni (Zimmerman)

– Body smaller, pronotum plus elytron ca. 3.02–3.06 mm. Elytron without subapical patch of white scales. .........................................................3

Figure 5. *Trigonopterus pembertoni* (Zimmerman), holotype. Dorsal habitus. Photo courtesy B.P. Bishop Museum.
3(2) Pronotum smooth, almost impunctate. Male mesotibia subapically with pre-micro larger than uncus .............. *T. obsidianus* Van Dam & Riedel, sp. n.

– Pronotum densely punctate. Male mesotibia subapically with uncus; pre-micro minute or absent.......................................................................................4

4(3) Body elongate. Metafemur subapically with stridulatory patch ....................

...................................................... *T. silaliensis* Van Dam & Riedel, sp. n.

– Body subovate. Metafemur without stridulatory patch .........................

................................................... *T. puncticollis* Van Dam & Riedel, sp. n.

Discussion

The absence of a record of a weevil genus from a Melanesian island is often difficult to interpret, i.e. it is usually unclear whether this is based on a true absence or on a lack of records. Prior to this study, *Trigonopterus* was unknown from New Britain. The four species described here represent four different clades of *Trigonopterus*, indicating that the oceanic island of New Britain has been colonized at least four times, and *T. pember-toni*, which occurs on neighboring New Ireland and represents the *T. oblongus*-group, brings the number of colonization events of *Trigonopterus* in the Bismarck Archipelago to five. Given the size, mountainous topography and tropical vegetation of New Brit-ain, it is likely that *Trigonopterus* has undergone some local speciation on the island, but this possibility requires further investigation.

Despite many days of searching for *Trigonopterus* in primary forest on New Brit-ain, the weevils were quite scarce in comparison with similar localities on the New-Guinean mainland. This scarcity may be due to the local conditions or seasonal ef-fects, as orographic precipitation formed early in the day and continued into the evening during our stay. The specimens’ habitat consisted of primary forest growing on a limestone karst.

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References


Ancient Himalayan wolf (Canis lupus chanco) lineage in Upper Mustang of the Annapurna Conservation Area, Nepal

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Abstract

The taxonomic status of the wolf (Canis lupus) in Nepal’s Trans-Himalaya is poorly understood. Recent genetic studies have revealed the existence of three lineages of wolves in the Indian sub-continent. Of these, the Himalayan wolf, Canis lupus chanco, has been reported to be the most ancient lineage historically distributed within the Nepal Himalaya. These wolves residing in the Trans-Himalayan region have been suggested to be smaller and very different from the European wolf. During October 2011, six fecal samples suspected to have originated from wolves were collected from Upper Mustang in the Annapurna Conservation Area of Nepal. DNA extraction and amplification of the mitochondrial (mt) control region (CR) locus yielded sequences from five out of six samples. One sample matched domestic dog sequences in GenBank, while the remaining four samples were aligned within the monophyletic and ancient Himalayan wolf clade. These four sequences which matched each other, were new and represented a novel Himalayan wolf haplotype. This result confirms that the endangered ancient Himalayan wolf is extant in Nepal. Detailed genomic study covering Nepal’s entire Himalayan landscape is recommended in order to understand their distribution, taxonomy and, genetic relatedness with other wolves potentially sharing the same landscape.

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Keywords
Himalayan wolf, wolf-dog clade, Canis lupus chanco, Trans-Himalaya, Annapurna Conservation Area, Nepal

Introduction

The presence of wolf (Canis lupus) in the Trans-Himalayan regions of Nepal has been reported for centuries (Hodgson 1847). The species is protected under the National Parks and Wildlife Conservation Act, 1973 of the Government of Nepal and listed as Critically Endangered in the National Red List (Jnawali et al. 2011). Although receiving federal protection status, wolves in this region have suffered heavy mortality, mainly due to retributive and preventive killing by livestock herders. Their imperiled status becomes all the more imminent for conservation in light of their evolutionary distinct origins from all other wolf lineages (Sharma et al. 2004, Aggarwal et al. 2007). Recent mitochondrial (mt) DNA analyses of wolves and dogs, from the Indian subcontinent, revealed the presence of three distinct wolf lineages in the region, which are basal and divergent to the globally distributed wolf-dog clade (Sharma et al. 2004). Further, the study also revealed that the Himalayan lineage of wolves (C. l. chanco) branches at an earlier point in the tree, and may have split as early as 0.8 to 1.5 million years ago (Sharma et al. 2004). Although recent studies reveal that the ancient Himalayan wolf lineage has been present in the Nepal Himalaya (Sharma et al. 2004), its current existence in Nepal is not certain. This is because documentation of the extant lineage was based on living wolf samples collected from Himachal Pradesh in India and DNA samples from Nepal were sourced entirely from museum specimens (Sharma et al. 2004).

During a recent survey in the Trans-Himalayan region of Upper Mustang, Annapurna Conservation Area, Nepal, wolves were encountered several times and their physical features were observed carefully (Figure 1). Characteristic features observed included distinct white coloration around the throat, chest, belly and inner part of the legs; woolliness of body fur; stumpy legs; unusual elongation of the muzzle, a muzzle arrayed with closely-spaced black speckles which extend below the eye on to the upper cheeks and ears; and smaller size compared to the European wolf (Gray 1863, Pocock 1941, Olsen and Olsen 1977). Based on these distinctive characteristics and skull morphology, Hodgson (1847) had classified this wolf as a separate species and called it C. laniger. Subsequently, Pocock (1941) grouped C. laniger with the Tibetan wolf subspecies (C. l. chanco). This taxonomic confusion regarding the identification and recognition of wolves from the Trans-Himalayan region of India and parts of Tibet has persisted for the last 165 years (Shrotriya et al. 2012). Aggarwal et al. (2007) claimed that the wolf ranging in the Trans-Himalayan landscape is a separate species or a subspecies of C. lupus, although the recognition of separate species or subspecies is pending more evidence from nuclear markers (Habib et al. 2013). Based on mtDNA sequence data, C. l. chanco was observed to be paraphyletic and consist of two divergent and parapatric lineages extant in the region (Sharma et al. 2004). The Tibetan Plateau lineage of C. l. chanco occurs in western and central Kashmir, Tibet,
Ancient Himalayan wolf (Canis lupus chanco) lineage in Upper Mustang...

Figure 1. A Himalayan wolf photographed in Upper Mustang of Annapurna Conservation Area, Nepal (29.17356°N, 84.13422°E; datum WGS84, elevation 5,050 m) during May 2014.

China, Mongolia and Russia, and falls under the widespread wolf-dog clade. On the other hand, the basal monophyletic Himalayan wolf mtDNA lineage of *C. l. chanco* is distinct from haplotypes in the wolf-dog clade, and is likely distributed from eastern Kashmir into eastern Nepal and Tibet.

In the present paper, we describe for the first time the extant mitochondrial lineage of wolves that inhabit the Trans-Himalayan region in Upper Mustang of Annapurna Conservation Area, Nepal, based on DNA extracted from fecal samples collected in the wild. We identified a novel mtDNA CR haplotype that clustered within the monophyletic Himalayan wolf clade of *C. l. chanco*.

Materials and methods

Field Sampling and Labwork

During October 2011, six fecal samples suspected to have originated from wolves were collected from Upper Mustang in the Annapurna Conservation Area of Nepal at an elevation ranging from 4,750 to 5,050 m asl (Figure 2). A small portion of the collected fecal samples were preserved in polypropylene vials using silica desiccant (Janêcka et al. 2008, Lovari et al. 2009). Fecal DNA was extracted using standard protocol (QIAamp
DNA stool kit, Qiagen Ag., Germany) and subsequent polymerase chain reaction (PCR) and DNA sequencing protocols followed methods outlined in Sharma et al. (2004). We PCR amplified the mtDNA control region (CR) locus using two sets of primer pairs, viz. - (i) ThrL15926 and DL-H16340 (Vila` et al. 1999) targeted ~ 440 base pairs (bp); (ii) IWD 220 F and IWD 220 R targeted a smaller region (~ 200bp), especially designed for amplification of “ancient” canid samples (Sharma et al. 2004).

**Sequence analysis**

MtDNA CR sequences were aligned and edited using Bioedit 5.0 (Hall 1999). Phylogenetic analyses were carried out using Bayesian and maximum likelihood procedures in MrBayes v3.2.2 (Ronquist et al. 2012) and MEGA 6 (Tamura et al. 2013) respectively. The Bayesian run length consisted of a total of 6 million Markov Chain Monte Carlo (MCMC) replicates, of which the first 1.5 million runs comprised the burn-in phase. Run convergence was assessed from the average standard deviation in split frequencies (< 0.01). Gaps were treated as missing data and not used for analysis in both MrBayes and MEGA. Trees were analyzed using the HKY (Hasegawa, Kishino and Yano, 1985), general time reversible (GTR, Tavare 1986), F81 (Felsenstein 1981) and mixed models.
of molecular evolution implemented in Mr Bayes. We analyzed the harmonic mean outputs of MrBayes runs using Bayes Factors (Kass and Raftery 1995) to obtain estimates of probabilities for the best model of molecular substitution for our data. The GTR substitution model with a gamma distributed rate variation and having a proportion of invariable sites (GTR + invgamma) was found to be the most likely model with the highest probability (P = 0.873) compared to all other models tested in this study (Suppl. material 1). The GTR + invgamma model was also implemented for maximum likelihood phylogeny construction in MEGA. Gaps were removed from analysis using the conservative ‘Complete Deletion’ option in MEGA. Confidence in estimated relationships was assessed using 1,000 bootstrap simulations. A maximum parsimony analysis was also conducted in MEGA for comparison. We compared our samples with corresponding sequences of other gray wolf lineages from the Indian-subcontinent and other regions, available at GenBank (see Suppl. material 2). The tree was rooted using sequence data from the maned wolf (Chrysocyon brachyurus) as outgroup, based on previously published phylogeny of canids (Lindblad-Toh et al. 2005). To estimate divergences and rates, we used MEGA to calculate mean Tamura-Nei genetic distances (with gamma shape parameter = 0.3) as used in previous studies on the same sequenced region in Himalayan wolves (Sharma et al. 2004). To examine genetic structuring among haplogroups, a median joining network tree (Bandelt et al. 1999) of CR haplotypes was constructed using the program network 4.613 (http://www.fluxus-engineering.com, Accessed 20 June 2015). Network calculations were carried out by assigning equal weights to all variable sites and with default values for the epsilon parameter (epsilon=0) in order to minimize alternative median networks. Gaps were treated as missing data and nucleotide alignment blocks containing indels were removed before analysis.

Results and discussion

We successfully obtained mtDNA control region sequences (~ 220 bp) from five (Table 1) out of a total of six fecal samples by using the shorter “ancient” DNA primer sets (see Suppl. material 3 for list of sequences). None of the six samples could be amplified using the larger (~ 440bp) primer pair. Such a result with scat samples is not unexpected, due to the already fragmented scat DNA extracts which render it difficult to amplify gene fragments larger than c. 200 to 300 bp (Janečka et al. 2008, Vynne et al. 2012). The species identity of one scat sample could not be established due to PCR failure. Out of the five successfully amplified fecal samples, the mtDNA sequences of four scats matched each other and were aligned within the monophyletic clade represented by the Himalayan wolf (Figure 3). The relationship was strongly supported with > 70% out of 1,000 bootstrap replicates in both maximum likelihood and maximum parsimony procedures (Figure 3A), and with > 0.75 posterior probability in Bayesian analysis (Figure 3B). Identical tree topologies were obtained in both the maximum likelihood and maximum parsimony analyses. Although there were minor differences in the resolution of few haplotypes in the maximum likelihood and Bayesian trees, the
<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>Species</th>
<th>Clade</th>
<th>Locality (datum WGS84); Altitude (m)</th>
<th>Area name</th>
<th>Haplotype</th>
<th>GenBank Acc #</th>
<th>Date</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2137</td>
<td><em>Canis lupus chanco</em></td>
<td>Himalayan wolf</td>
<td>N29°12'14.3&quot;, E084°8'24.8&quot;; 5020</td>
<td>Yarsa</td>
<td>HW-F</td>
<td>KT321360</td>
<td>11.10.2011</td>
<td>Madhu Chetri</td>
</tr>
<tr>
<td>D2138</td>
<td><em>Canis lupus chanco</em></td>
<td>Himalayan wolf</td>
<td>N29°12'58.4&quot;, E084°6'32.1&quot;; 4740</td>
<td>Yarsa</td>
<td>HW-F</td>
<td>KT321360</td>
<td>11.10.2011</td>
<td>Madhu Chetri</td>
</tr>
<tr>
<td>D2139</td>
<td><em>Canis lupus chanco</em></td>
<td>Himalayan wolf</td>
<td>N29°10'24.2&quot;, E084°8'3.5&quot;; 5050</td>
<td>Dharkeko pass</td>
<td>HW-F</td>
<td>KT321360</td>
<td>12.10.2011</td>
<td>Madhu Chetri</td>
</tr>
<tr>
<td>D2143</td>
<td><em>Canis lupus chanco</em></td>
<td>Himalayan wolf</td>
<td>N29°10'24.2&quot;, E084°8'3.5&quot;; 5050</td>
<td>Dharkeko pass</td>
<td>HW-F</td>
<td>KT321360</td>
<td>12.10.2011</td>
<td>Madhu Chetri</td>
</tr>
<tr>
<td>D2140</td>
<td><em>Canis familiaris</em></td>
<td>Domestic dog</td>
<td>N29°13'25.5&quot;, E084°6'10.6&quot;; 4740</td>
<td>Dhalung</td>
<td>ID-H</td>
<td>KT321361</td>
<td>14.10.2011</td>
<td>Madhu Chetri</td>
</tr>
</tbody>
</table>

**Table 1.** Specimen ID, location and area name with GenBank account number of the sequenced samples.
Ancient Himalayan wolf (Canis lupus chanco) lineage in Upper Mustang...

Figure 3. Phylogenetic trees constructed using 229 bp of aligned CR sequence data. The values at nodes correspond to A bootstrap support > 50% in maximum likelihood (ML) and maximum parsimony (MP) analyses; and B Bayesian posterior probability > 0.50. Scat samples sequenced in this study are highlighted in bold. Four samples (D2137, D2138, D2139 and D2143) represented a novel haplotype HWF within the Himalayan wolf clade, while a fifth sample (D2140) matched with existing domestic dog haplotypes.
overall relationship was consistent with the Himalayan wolf haplotypes forming a basal clade to all other wolf lineages. The tree topology suggests that these four matched samples are derived individuals of the ancient Himalayan lineage of *C. l. chanco* and not the Tibetan wolf lineage of *C. l. chanco* which falls within the widespread wolf-dog clade. Additionally, the median-joining haplotype network analysis indicated that these scat sequences formed part of the Himalayan wolf lineage (Suppl. material 4), further lending support to the results of phylogenetic analyses.

The sequences of these four scat samples which clustered within the monophyletic Himalayan wolf clade are new and not identical to haplotypes identified previously in GenBank. We therefore designated this novel haplotype HWF, in line with the five existing HW (A to E) haplotypes identified previously by Sharma et al. (2004). Mean Tamura-Nei genetic distance between HWF and the rest of the Himalayan wolf haplotypes, corrected for intra-clade variation, was very low (1.2 ± 0.9 %), compared to significant divergence between members of the wolf-dog lineage of *C. l. chanco* (10.5 ± 5.5 %), and other wolf (9.2 ± 4.8 %) and dog lineages (10.1 ± 5.5 %). HWF differed from the nearest haplotype, HWC by two base substitutions and by three base substitutions from HWA (Suppl. material 3 and 4). Although not detected in our sampled sequences, both HWC and HWA haplotypes were previously reported in museum samples from Nepal (Sharma et al. 2004).

The sequence of a fifth scat sample fell within the domestic dog clade (*C. familiaris*). BLAST analyses in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) indicated 100% sequence match to many well represented domestic dog breeds from different regions of the world (see the Blast report pdf file in Suppl. material 5). Notably, these included the Indian domestic dog haplotype, ID-H, previously detected in Himalayan Bhotia sheepdogs (Sharma et al. 2004), Tibetan mastiffs from China, domestic dogs from Europe and the Middle East, and also a wolf individual from Iran carrying domestic dog introgressed mtDNA (Aghbolaghi et al. 2014). Given the proximity of sampled sites and niche overlap between wolves and dogs in the area, hybridization between the two species cannot altogether be ruled out. However, our sampling and analytical methods were inadequate for this purpose, and high resolution genome wide investigations using bi-parentally inherited markers are required for such hybridization studies.

The results of molecular analysis support our initial assumption, based on morphological observations, that the wolves found in Upper Mustang region of the Annapurna Conservation Area, Nepal, include individuals that belong to the genetically distinct and ancient Himalayan wolf clade (Figures 1 and 3). Although, it is plausible that both the Himalayan and Tibetan wolf (wolf-dog clade) lineages of *C. l. chanco* share the same landscapes (Sharma et al. 2004), we did not detect any individuals belonging to the latter clade. The remoteness of the terrain compounded by low population density of wolves in the area, made it difficult to locate and collect scats. However, despite our limited sampling we were able to detect four scats that originated from individuals aligned to the Himalayan wolf-dog clade of *C. l. chanco*. Given the close proximity of the sampled locations and absence of microsatellite genotypic information in our data, we are unable to confirm whether the sequences of these four scats originated from the
same or from different individuals. Future noninvasive fecal sampling studies should cover the entire Himalayan landscape of Nepal so as to understand the distribution of gray wolf lineages in the region. Such surveys will also provide information on their population status and conservation threats.

As part of the ongoing long term ecological research on wolves, both formal and informal interviews with herders, livestock owners, nomads and village elite were conducted in order to understand the status of human-wolf conflict, local attitudes and perceptions. Formal interview involves semi-structured questionnaire survey (n=354) which covers all the potential areas of wolf distribution in Mustang and Manang Districts of Annapurna Conservation Area. Informal interview (n=61) was mainly through discussion when herders were encountered while herding their livestock or while visiting their herding camps/corrals. Our preliminary assessment revealed that local communities persecuted wolves mainly in retaliation for livestock depredation. In some parts of the conservation area, livestock depredation from wolves was found to be a cause of concern for local livelihoods. These genetically distinct Himalayan wolves deserve special conservation attention, at the same time that the conservation of this species in a context of human-wildlife conflict is challenging. A species action plan needs be formulated that develops mechanisms to minimize conflict, and strategies for motivating local communities towards wolf conservation.

Acknowledgements

We are grateful to the National Trust for Nature Conservation’s Annapurna Conservation Area Project for providing us the research permission to carry out field work. Long term ecological study of the wolf in Nepal has been funded by USAID/Hariyo Ban Nepal Ko Dhan Program and Hedmark University of Applied Sciences, Norway. We are also thankful to the Wildlife Institute of India for collaborating on this research and providing an access to database of sequences. We are grateful to Broughton Coburn (Wyoming, USA) for assistance with editing. We would like to thank all the field staff of National Trust for Nature Conservation’s Annapurna Conservation Area Project and local communities who were directly and indirectly involved in the field work. We also wish to thank both reviewers, especially Rob Fleischer at the Center for Conservation and Evolutionary Genetics (Smithsonian Institution, USA), whose insightful and constructive comments immensely improved the manuscript.

References


Ancient Himalayan wolf (Canis lupus chanco) lineage in Upper Mustang...


Supplementary material 1

Best model selection for Bayesian analysis
Authors: Madhu Chetri, Yadavendra V. Jhala, Shant R. Jnawali, Naresh Subedi, Maheshwar Dhakal, Bibek Yumnam
Data type: DOCX file
Explanation note: Estimating the best model of molecular substitution inferred using Log Bayes Factors (LBF) from Bayesian posterior distributions in Mr Bayes. Rate variation for tested models - gamma distributed rate variation across sites (gamma); gamma distributed with proportion of invariable sites (invgamma); rate variation with proportion of invariable sites (propinv); equal rate variation across sites (equal).
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Supplementary material 2

Genebank sequences analysed in this study
Authors: Madhu Chetri, Yadavendra V. Jhala, Shant R. Jnawali, Naresh Subedi, Maheshwar Dhakal, Bibek Yumnam
Data type: DOCX file
Explanation note: GenBank accession numbers for sequences analyzed in this study.
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Supplementary material 3

Aligned CR sequences of selected samples of wolves and dogs from GenBank, with the obtained scat samples
Authors: Madhu Chetri, Yadendradev V. Jhala, Shant R. Jnawali, Naresh Subedi, Maheshwar Dhakal, Bibek Yumnam
Data type: DOCX file
Explanation note: Aligned CR sequences of selected samples of wolves, dogs from GenBank, with scat samples obtained in this study. Numbers refer to mtDNA nucleotide positions referenced with respect to the complete mtDNA genome of gray wolf (GenBank Accession No. KF857179). Scat sequences D2137, D2138, D2139 and D2143 match each other differs from known Himalayan wolf haplotypes by at least two substitutions, while D2140 completely matches domestic dog and a wolf sequence in GenBank.
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Supplementary material 4

Median-joining networks of Himalayan wolf and related wolf and dog clades
Authors: Madhu Chetri, Yadendradev V. Jhala, Shant R. Jnawali, Naresh Subedi, Maheshwar Dhakal, Bibek Yumnam
Data type: TIF file
Explanation note: Median-joining networks of Himalayan wolf and related wolf and dog clades. Golden jackal and Ethiopian wolf haplotypes are shown for comparison. Circle size and branches are proportional to sampled haplotype frequency and number of nucleotide mutation steps among haplotypes, respectively. Branch numbers refer to mutation steps separating individual haplotypes. Scat samples sequenced in this study are represented by arrows falling within the Himalayan wolf (HWF) and Indian feral dog (IDH) haplotypes. Nomenclature for the African wolf follows Koepfli et al. (2015).
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Supplementary material 5

Blast report of sample D2140
Authors: Madhu Chetri, Yadwendrad Jhala, Shant R. Jnawali, Naresh Subedi, Maheshwar Dhakal, Bibek Yumnam
Data type: PDF file
Explanation note: Blast search result for D2140 scat sequence.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Iranian terrestrial isopods of the family Cylisticidae Verhoeff, 1949 with a description of a new species (Isopoda, Oniscidea)

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Abstract
In the present study, the terrestrial isopods of the family Cylisticidae in Iran are investigated. Geographical distributions of two formerly reported species from Iran, namely Cylisticoides angulatus Schmalfuss, 2003 and C. rotundifrons (Schmalfuss, 1986), are expanded. Cylisticus masalicus sp. n. is described and its diagnostic characters are figured.

Keywords
Oniscidea, Cylisticidae, Cylisticus, Cylisticoides, new species, Iran

Introduction
Verhoeff (1949) established the subfamily Cylisticinae for the genus Cylisticus Schnitzler, 1853 in the family “Porcellionidae”. Strouhal (1953) did not accept the recognition of Cylisticinae as a separate group and proposed the inclusion of all forms with five pairs of lungs in the subfamily Trachelipinae. Vandel (1962) followed Verhoeff’s view and actually raised Cylisticidae to family level while Schmalfuss (2003a) questioned the validity of the family.

Members of Cylisticidae are characterized by: strongly convex body; exoantennal conglobation ability, though the head and telson are poorly adapted to conglobation; pereon tergite I without modifications or with grooves to receive the antennae; head with developed lateral and median lobes; supra-antennal line absent; antenna long;
flagellum with two articles; pleopod exopodites I-V with wrinkled, partly covered, “Trachelipus-type”, or with internal, covered, “Porcellio-type” lungs (Schmalfuss 1992, 2003a; Schmidt 2003). There are no clear synapomorphies defining the taxon.

Cylisticidae species are distributed from central Europe to central Asia. According to the world catalogue of terrestrial isopods (Schmalfuss 2003b), the family comprises five genera, namely Cylisticoides Schmalfuss, 2003, Cylisticus Schnitzler, 1853, Lepinisticus Manicastri & Taiti, 1983, Parcylisticus Verhoeff, 1943, and Troglocylisticus Ferrara & Taiti, 1983. Schmalfuss (2003b) included Cylisticoides in the family Cylisticidae with a question mark, since he believed that the superficial similarity of the genus Cylisticoides with the other genera might be due to convergence.

Schmalfuss (1986) recorded the genus Cylisticus from Iran, based on three female specimens, as Cylisticus sp. I. In the same contribution, he also described Cylisticus rotundifrons which was later (Schmalfuss 2003a) transferred to the new genus Cylisticoides. He also named another female specimen as Cylisticus sp. II, which was later described as Cylisticoides angulatus (Schmalfuss 2003a).

In the present study, new records for Cylisticoides angulatus and C. rotundifrons in Iran are presented and Cylisticus masalicus sp. n. is described.

Material and methods

The material examined herein was collected in Iran (Fig. 1), mostly from May 2014 to September 2015. The specimens were collected by hand and preserved in 96% ethanol. Drawings were made using a drawing tube on a Nikon Y-IDT compound microscope. Type material of the newly described species is deposited in the Zoological Museum, University of Tehran (ZUTC), the Iranian Research Institute of Plant Protection (IRIPP) and in the personal collection of the author (PCGMK).

Taxonomy

Order Isopoda Latreille, 1817
Suborder Oniscidea Latreille, 1802
Family Cylisticidae Verhoeff, 1949

Cylisticoides rotundifrons (Schmalfuss, 1986)


Material examined. Amol to Chamestan, Belvich village, 36°28.3’N, 52°10.0’E, elev. 60 m, 29 July 2014, leg. G.M. Kashani, two females (PCGMK 1960); Neka to Behshahr, Pasandrez forests, 36°40.0’N, 53°36.7’E, elev. 300 m, 31 July 2014, leg. G.M. Kashani, one female (PCGMK 1998).
Iranian terrestrial isopods of the family Cylisticidae Verhoeff, 1949...

Distribution. N Iran.

Remarks. Schmalfuss (1986) described *Cylisticus rotundifrons* from a female specimen collected at 12 km NE Zirab, Iran. He also named two females from Noor district as *Cylisticus aff. rotundifrons*. Schmalfuss (2003a) transferred the species to the genus *Cylisticoides*. In the present study, no male specimen was found either. Despite been relatively widely distributed in northern Iran (Fig. 1), *C. rotundifrons* does not seem to be common. Though no male is known for the species, it can be readily distinguished from *C. angulatus*, the only congeneric species, from the rounded (vs. angled) postero-lateral margin of pereon-tergite I and the lack of a ridge on lateral margin of pereon-tergite I (present in *C. angulatus*). Based on current knowledge, *C. rotundifrons* is restricted to northern Iran.

*Cylisticoides angulatus* Schmalfuss, 2003

Material examined. Gorgan, Naharkhoran district, 36°46.8’N, 54°27.8’E, elev. 430 m, 1 August 2014, leg. G.M. Kashani, eight males, eight females and four juv. (PCGMK 2010); 15 km S Jalin, 36°42.5’N, 54°35.3’E, elev. 900 m, 1 August 2014, leg. G.M. Kashani, one male, three females and seven juv. (PCGMK 2016); Ramian to Shahrood, 36°52.1’N, 55°13.4’E, elev. 1375 m, 2 August 2014, leg. G.M. Kashani, one male, three (PCGMK 2021); Poonel to Sangdeh, 37°32.0’N, 48°56.7’E, elev. 420 m, 14 August 2014, leg. G.M. Kashani, five females and three juv. (PCGMK 1800); Poonel to Sangdeh, 3 km to Zendaneh, 37°32.2’N, 48°45.1’E, elev. 1500 m, 14 August 2014, leg. G.M. Kashani, three males, four females and two juv.. (PCGMK 1801); Fooman, Ghaleh Roodkhan, 37°04.5’N, 49°14.9’E, elev. 330 m, 15 August 2014, leg. G.M. Kashani, six males, two females and thirteen juv. (PCGMK 1807); Shaft, Si-ahmazgi, 37°01.3’N, 49°16.4’E, elev. 400 m, 14 August 2014, leg. G.M. Kashani, four males, four females (PCGMK 1810); Someh-Sara, 37°17.7’N, 49°18.6’E, elev. 15 m, 16 August 2014, leg. G.M. Kashani, five males, two females and three juv. (PCGMK...
1821); Fooman to Roodbar, 37°10.8'N, 49°33.4'E, elev. 50 m, 17 August 2014, leg. G.M. Kashani, one female (PCGMK 1822); Saravan, 37°07.8'N, 49°38.9'E, elev. 60 m, 17 August 2014, leg. G.M. Kashani, six males and one juv. (PCGMK 1823); Kiashahr port, 37°25.6'N, 49°57.6'E, 18 August 2014, leg. G.M. Kashani, one female (PCGMK 1837); Siahkal to Deylaman, Loonak village, 37°03.4'N, 49°53.7'E, 19 August 2014, leg. G.M. Kashani, one female and three juis. (PCGMK 1842); Klar Dasht to Abbasabad, 36°37.5'N, 51°06.4'E, elev. 400 m, 12 September 2014, leg. G.M. Kashani, one subadult (PCGMK 1885); Ramsar to Javaheerdeh, 36°54.6'N, 50°36.7'E, elev. 170 m, 14 September 2014, leg. G.M. Kashani, two males and eight females (PCGMK 1891); Ramsar to Javaheerdeh, 36°52.6'N, 50°33.4'E, elev. 730 m, 14 September 2014, leg. G.M. Kashani, four males, one female and eighteen juvis. (PCGMK 1896); Masooler, 19 July 2004, leg. G.M. Kashani, two females (PCGMK 1174); Galikesh to Bojnurd, Golestan National Park, 37°23.0'N, 55°50.7'E, 6 May 2008, leg. G.M. Kashani, two females (PCGMK 1180); 6 km S Shirgah, 36°15.9'N, 52°53.9'E, elev. 210 m, 8 June 2015, leg. G.M. Kashani, two females (PCGMK 2088); Shirgah, 36°16.9'N, 52°53.2'E, elev. 240 m, 8 June 2015, leg. G.M. Kashani, three males and six females (PCGMK 2090); Amol to Chamestan, Belvich village, 36°28.3'N, 52°10.0'E, elev. 60 m, 3 September 2015, leg. G.M. Kashani, twelve males and eighteen females (PCGMK 2102); 17 km S Amol, 36°22.0'E, elev. 500 m, 4 September 2015, leg. G.M. Kashani, five males and four females (PCGMK 2104).

**Distribution.** SE Azerbaijan; N Iran.

**Remarks.** Schmalfuss (2003a) established a new genus and species for specimens collected from southeastern Azerbaijan, namely *Cylisticoides angulatus*. He also assigned one female specimen collected from Dashtis Nazir, Iran, previously named as *Cylisticus* sp. II (Schmalfuss 1986), to this species.

In the present study, *C. angulatus* was collected at a broad range of localities in northern Iran (Fig. 1). The preferred habitat for this species seems to be the bark of decaying trees in old forests.

### **Cylisticus masalicus** sp. n.

http://zoobank.org/6A53EAA9-369C-4962-ABB8-E65E7B0E1DA7

**Material examined.** Holotype: male, 11 mm, IRAN, Gilan, Masal, 37°19.0'N, 48°59.0'E, elev. 600 m, 19 March 2014, leg. G.M. Kashani (ZUTC 5786).

Paratypes: same data as holotype, one male and two females (ZUTC 5787); same data as holotype, one male and one female (IRIPP Iso-1063); same data as holotype, three males and four females (PCGMK 1749); Gachsar to Marzanabad, 5 km to Dozdband, 36°16.2'N, 51°14.6'E, 26 July 2014, leg. G.M. Kashani, one female (PCGMK 1921); Noor to Kojour, 36°26.2'N, 51°53.3'E, elev. 790 m, 28 July 2014, leg. G.M. Kashani, one female (PCGMK 1942); Noor to Kojour, Kodir village,
36°26.4’N, 51°51.6’E, elev. 1435 m, 28 July 2014, leg. G.M. Kashani, one female and one juv. (PCGMK 1946); Kojour to Galandrood, 36°26.7’N, 51°50.7’E, elev. 1480 m, 28 July 2014, leg. G.M. Kashani, one male (PCGMK 1952); Poonel to Sangdeh, 37°33.4’N, 48°41.5’E, elev. 2200 m, 14 August 2014, leg. G.M. Kashani, one male and one female (IRIPP Iso-1062); Poonel to Sangdeh, 37°33.4’N, 48°41.5’E, elev. 2200 m, 14 August 2014, leg. G.M. Kashani, four males, five females and one juv. (PCGMK 1803); 29 km to Asalem, 37°37.5’N, 48°48.6’E, elev. 2220 m, 14 August 2014, leg. G.M. Kashani, four females (PCGMK 1804); 10 km to Shaft, 37°06.2’N, 49°23.8’E, elev. 80 m, 15 August 2014, leg. G.M. Kashani, one female (PCGMK 1813); Siahkal to Deylaman, 10 km to Deylaman, 36°56.2’N, 49°51.8’E, elev. 1500 m, 19 August 2014, leg. G.M. Kashani, one male (PCGMK 1845); Boomehen to Amol, Ploor village, 35°50.9’N, 52°03.2’E, elev. 2200 m, 11 September 2014, leg. G.M. Kashani, two females (PCGMK 1846); Tonekabon, Darbar village, 36°42.7’N, 50°50.7’E, elev. 220 m, 13 September 2014, leg. G.M. Kashani, three females and two males (PCGMK 1882); Galesh-Mahalleh to Jannat-Roodbar, 36°49.3’N, 50°41.3’E, elev. 370 m, 13 September 2014, leg. G.M. Kashani, one female (PCGMK 1888); Ramsar to Javaherdeh, 36°52.5’N, 50°33.3’E, elev. 770 m, 14 September 2014, leg. G.M. Kashani, one male and one female (PCGMK 1893); Amlash, Khorma village, 37°04.5’N, 49°58.9’E, elev. 270 m, 14 September 2014, leg. G.M. Kashani, one male (PCGMK 1902); Rahim-Abad to Ziaz, 36°56.5’N, 50°16.5’E, elev. 220 m, 15 September 2014, leg. G.M. Kashani, two males and one female (PCGMK 1912).

**Diagnosis.** Cephalon with well developed quadrangular lateral lobes; median process pointed upwards; male pereopod VII ischium subrectangular; male pleopod endopodite I with apical part slightly bent outwards, bearing some setae.

**Description.** Maximum length of both male and female, 15 mm. Color slaty gray with the usual pale muscle spots (Fig. 2A, B). Exoantennal conglobation (Fig. 2A) and body semi-circular in cross section. Cephalon with well developed quadrangular lateral lobes; median process pointed upwards, not surpassing lateral lobes in frontal view; vertex smooth, eyes with 20 to 22 ommatidia (Fig. 3A, B). Antenna long and slender, bent on the back when conglobating; fifth article of peduncle slightly longer than flagellum, with length:width ratio 7:1; flagellum with two articles of the same size (Fig. 3D).

![A](image1.png) ![B](image2.png)

**Figure 2.** *Cylisticus masalicus* sp. n.; A conglobated B active.
Figure 3. *Cylisticus masalicus* sp. n., male, paratype. **A** cephalon dorsal view **B** cephalon frontal view **C** left side of the body showing the position of noduli laterales **D** antenna **E** telson and uropods **F** pereopod I and enlarged carpus and dactylus **G** pereopod VI and enlarged basis **H** pereopod VII and enlarged ischium. Scale: 1 mm (**A–C**); 0.5 mm (**D–H**).

Pereon smooth. Pereonite I with angular concavity on posterolateral margin. Noduli laterales on pereonite IV more than twice distant from the lateral margins than those on pereonite III (Fig. 3C).
Pereopod I ischium triangular, carpus with depression on rostral surface equipped with slender scales, dactylus with one dactylar and one ungual seta (Fig. 3F).

Pleon as broad as pereon (Fig. 3E). Telson triangular, with concave sides and rounded apex, surpassing uropod-protopodites. Uropod-exopodites short, 2/3 as long as telson (Fig. 3E). Pleopod exopodites I–V with polyspiracular internal lungs (Fig. 4B–F).

Male: Pereopods I–VII merus and carpus with brushes of trifid setae, less dense in posterior ones (Fig. 3F–H). Pereopod VI ischium on sternal margin with three long spiny setae medially and three long spiny setae distally (Fig. 3G), pereopod VII ischium subrectangular, ventral side with a hairy brush of small setae, medially with six and distally with four long spiny setae (Fig. 3H). Pleopod endopodite I straight with apical part slightly bent outwards equipped with some short setae (Fig. 4A); exopodite I with rounded hind lobe, outer margin with a row of small setae (Fig. 4B, C).
Pleopod endopodite II longer than exopodite; exopodite triangular with a line of setae on outer margin (Fig. 4D). Pleopod exopodites III–V as in Fig. 4E–G.

**Etymology.** The name of the species is after the type locality, the rain forests around Masal.

**Distribution.** N Iran.

**Remarks.** In the examination of a collection of terrestrial isopods from northern Iran, Schmalfuss (1986) reported the genus *Cylisticus* for the first time based on three female specimens, which he cited as *Cylisticus* sp.I. This was the sole account for this genus up to now. In the present study, *C. masalicus* sp. n. is described. It has a broad distribution in the rain forests of the country (Fig. 5). According to the illustrations presented by Schmalfuss (1986) for the specimens named *Cylisticus* sp.I. and their collecting localities that lies inside the geographical range of the species (Fig. 5), those specimens presumably belong to *C. masalicus* as well.

*Cylisticus masalicus* sp. n. differs from other species of the genus by the straight apex of the male pleopod endopodite I which is also common in *Parcylisticus* species. The new species, however, is well assignable to the genus *Cylisticus* for its smooth body surface and short uropod exopodites. This species is similar to *C. birsteini* Borutzky, 1961 and *C. iners* Budde-Lund, 1880 but differs from the former in the shape of the male pleopod endopodite I, and from the latter in the position of noduli laterales and the shape of telson.

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References


