

Ornithodoros (Ornithodoros) huajianensis sp. nov. (Acari, argasidae), a new tick species from the Mongolian marmot (*Marmota bobak sibirica*), Gansu province in China

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ABSTRACT

A new argasid species, belonging to the subgenus *Ornithodoros*, namely, *Ornithodoros (Ornithodoros) huajianensis* was described for the first time based on the females, males and nymphs. The morphological features of each stage in the life cycle are unique, making identification easy, but are similar to other species of the subgenus *Ornithodoros*. The new species was diagnosed by the broad rectangular tongue and triangular tongue-shaped posterior lip in the male genital apron, a shallow camerostome with definite folds and smaller mammillae with single seta mixed with larger ones in nymph and adults. The new species had been collected from the Mongolian marmots *Marmota bobak sibirica* in Huajian village, Gulang county, Gansu province, China. Data on the phylogenetic position, hosts and geographic distribution are also provided.

1. Introduction

Argasid ticks comprise 216 valid species all over the world currently (Dantas-Torres, 2018; Mans et al., 2019), whose taxonomic status is still uncertain with more disputes on assignment of species to, and relationships between genera (Guzmán-Cornejo et al., 2016). Historically, the controversial issues had been argued among 4 competing classification schemes, including the Soviet scheme (Filippova, 1966; Pospelova-Shtrom, 1946, 1950; 1969), the American scheme (Sonenshine et al., 1966; Clifford et al., 1964; Hoogstraal, 1955, 1957; 1985; Hoogstraal and Kohls, 1966), the French scheme (Camicas and Morel, 1977; Camicas et al., 1998) and the morphological cladistic scheme (Klompen and Oliver, 1993). The confusion and disagreement of the 4 schemes in argasid systematics is mainly caused by the different determinations for the overall similarity of homoplasious features to provide coherent systematic clustering within taxonomic rank (Estrada-Pena et al., 2010, 2017). The morphotaxonomic approaches retain mostly in the phenetic analysis, rather than the phylogenetic concept of monophyletic clad (Burger et al., 2014). The known morphological characters of Argasidae, presented as high level of biodiversity, are not stable enough to produce a reliable guideline to differential diagnosis in

subgenera level, or higher rank universally acknowledged by most taxonomists (Burger et al., 2014). Consequently, about 137 argasid species have been assigned to more than one genus by different researchers (Guglielmone et al., 2010; Nava et al., 2010; Venzal et al., 2012). Recently, mitochondrial genome sequences had been shown to be potential to resolve the controversial phylogenetic relationships within soft tick lineages (Burger et al., 2014). A new designation for argasid genera and their subgenera was proposed to provide a better resolution for the species- and genus-level phylogeny within Argasidae family based on mitochondrial evolutionary evidences (Mans et al., 2019). The new classification scheme was shown corresponded broadly with the morphological cladistic analysis of Klompen and Oliver (1993), which raised *Carios* as a valid genus in subfamily Ornithodorinae for species in the genera *Antricola*, *Argas*, *Ornithodoros* and *Nothoapis*. When subgenera of *Ornithodoros*, *Ornamentum*, *Microargas* *Hoogstraal and Kohls*, 1966, *Pavlovskyella* *Pospelova-Shtrom* 1950 and former *Theriodoros* were embraced, genus *Ornithodoros* would be clustered within a monophyletic clade and be shown as a sister lineage of genera *Carios*, *Chiropterargas*, and *Obobius* (Mans et al., 2019). Currently, genus *Ornithodoros* comprises 47 valid species and represents the subfamily Ornithodorinae with definite and consensus

List of Abbreviations: O, *Ornithodoros*; SEM, Scanning electron microscopy; IOZ, Institute of zoology; Chinese academy of sciences; AMMSC, Academy of military medical sciences; ICZN, International code of zoological nomenclature; LSID, The life science identifier

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features derived from morphological, molecular phylogenetic analysis and other integrative approaches (Filippova, 1963; Nava et al., 2009; Guglielmone et al., 2010; Dantas-Torres, 2018; Mans et al., 2019). Most of the known *Ornithodoros* species occur in arid or semiarid areas, where ticks can find shelters in caves, rodent burrows and bird nests, but also in cracks and crevices of huts and shelters for people and domestic animals (Jongejan and Uilenberg, 2004). Once encountered, *Ornithodoros* ticks would pursue to attach and feed on human, cattle or other animals available after a long term hungry and cause serious damages even death to human or animal victims due to blood loss, irrigation or various tick-borne diseases e.g. African swine fever, Crimean Congo Hemorrhagic Fever, Karshi virus or Langat virus associated encephalitis and human relapsing fever (Manzano-Román et al., 2012). Therefore, the genus *Ornithodoros* appears to be the most hazardous to human and animals' health in the family Argasidae.

Chinese argasid fauna is far from a well-characterized category due to the limited investigations (Chen et al., 2010; Estrada-Peña et al., 2017). Incidentally, only 13 argasid species were recorded and assigned to two subfamilies (Chen et al., 2010). Of which, only 2 *Ornithodoros* species (*O. (Pavlovskyella) tartakovskyi*, *O. (P.) papillipes*) and 4 *Carios* species, *Carios (Carios) verspertilionis*, *C. (C.) pusilla*, *C. (C.) sinensis* (Jeu and Zhu (1982) and *C. (Alectorobius) capensis* were documented in the subfamily Ornithodorinae. The rest 7 species were assigned to genus *Argas* in the subfamily Argasinae (Burger et al., 2014; Wen and Chen, 2016). From viewpoints on diverse landscapes and climate zones within Chinese territory, these would not be the whole nature of Chinese argasid fauna, the discovery and descriptions of *C. (C.) sinensis* (Jeu and Zhu, 1982, 1985), *A. (Argas) assimilis* (Teng and Song, 1983) and *A. (A.) beijingensis* (Teng, 1983) from bat and avian hosts in China had put in evidences that the diversity of argasid ticks in China is probably underestimated and remained to be fully discovered. Fortunately, we discovered a new tick species of subgenus *Ornithodoros* from the Mongolian marmots in Gulang county, Gansu province, China. The aim of the present paper is to add new data to the Chinese argasid fauna, with the new record subgenus and the description of the new tick species belonging to genus *Ornithodoros*. A morphological account is provided with nymph, female and male specimens. Phylogenetic analysis based on the 16S rRNA gene sequences supports the placement of the new species within a large clad that includes other species associated *Ornithodoros*. However, the new species seems to represent an independent lineage within the subgenus *Ornithodoros*.

2. Materials and methods

2.1. Tick collection

On June 6, 1991, 3 female Mongolian marmots were captured in Huajian village, Gulang county, Gansu province by live traps. After anesthetization with CO₂, *Ornithodoros* ticks were collected from the Mongolian marmots and immediately placed in labeled vials containing 70% ethanol. The *Ornithodoros* ticks were transferred to the laboratory for further species identification and description.

2.2. Morphological study

Representative tick specimens were cleaned with ultrasound (20 kHz) using distilled water and commercial detergent in a proportion of 9:1 and then prepared for scanning electron microscopy as described previously (Corwin et al., 1979). Specimens of females, males, and nymphs were identified using morphological keys and original species descriptions of *Ornithodoros* spp. (Nuttall et al., 1908; Matheson, 1935, 1941; Cooley and Kohls, 1941, 1944; Theiler and Hoogstraal, 1955; Kohls et al., 1965, 1969; Jones and Clifford, 1972; Nava et al., 2012, 2013) under a Leica DM2500 stereomicroscope. Morphological terminology generally follows that of Walton (1962) and measurements were made in millimeters (mm) being expressed as mean followed by

standard deviation and range within parentheses. All the micrographs were produced through light and scanning electron microscopy (SEM) in the Electron Microscopy Laboratory, in Beijing Institute of Microbiology and Epidemiology, State Key Laboratory of Pathogen and Biosecurity of China.

2.3. Molecular procedure

Genomic DNA was extracted respectively from one female, and 1 male and 1 nymph using BlackPREP Tick DNA/RNA kit (Analytic Jena) under the guidance of user's manufacture. The extracted DNA samples were then subjected to PCR targeting fragments of approximately 460 base pairs of the mitochondrial 16S rDNA (Black and Piesman, 1994; Burger et al., 2014). The products were purified and sequenced using the same primers as used in the PCR. These sequences were aligned using Clustal X and adjusted manually using the MEGA version 6.0 with sequences previously determined for other *Ornithodoros* species available in GenBank (the accession numbers of all the sequences are shown in the resulting phylogenetic tree). The phylogenetic tree was inferred by means of the maximum parsimony (MP) method using MEGA version 6.0, with 1000 replicates of random addition taxa and TBR branch swapping. All positions were equally weighted and Bayesian analysis was performed using MrBayes v3.1.2 with 1,000,000 replicates (Ronquist and Huelsenbeck, 2003). The first 25% of the trees represented burn-in, and the remaining trees were used to calculate Bayesian posterior probability.

3. Results

1. Description of *Ornithodoros (Ornithodoros) huajianensis* Sun, Xu, Liu and Wu.

3.1. Diagnosis

Adults may be distinguished by a combination of (1) anterior and posterior dorsal mammillae subequal size, bulbous shape, moderately spaced, smaller mammillae with single seta mixed in larger ones (2) capitulum enveloped in a shallow camerostome, (3) eyes absent, (4) female genital apron half-moon to gentle crescent shape, male genital apron with broad rectangular tongue and triangular posterior lip, (5) intermammillary space with cell patterned ridges indistinct, (6) tarsus I proximal lobe tall, thick, rounded to curved, (7) tarsus I middle lobe tall, moderate width, sub-rectangular to slightly curved, (8) tarsus I proximodistal lobe wide, very short, (9) tarsus I distodistal lobe equilateral sub-triangular, short. Nymphs may be distinguished by (1) eyes absent, (2) anterior and posterior dorsal mammillae sub-equal size, smaller mammillae with single seta mixed in larger ones (3) posterior mammillae bulbous, (4) basis capitulum short, wide sub-rectangular, and (5) hypostome moderate thickness.

3.2. Female

Measurement and descriptions were conducted based on 4 females (Fig. 1 ~ 2; supplemental Fig. S1 ~ S4).

Body: Body generally rectangular-oval, widely rounded at anterior and posterior ends; 6.15 ± 0.6 (6.09–6.21) mm in length (from anterior end to posterior body margin), 4.16 ± 0.9 (4.07–4.25) in maximum width (Fig. 1, Supplemental Fig. S1).

Dorsum: Color light brown in unfed, preserved specimens; Dorsoventral groove between legs III and IV; Margin thick and not clearly defined, integument leathery without hood (Fig. 1); Mammillae generally opaque with indistinct transparent center, moderate sized mixed with much smaller ones, on which each bears a single seta, blunt, occasionally clubbed. Anterior mammillae moderate size, moderately separated, circular to oval shape, somewhat raised, flat apically; central mammillae moderate size, moderately separated, circular shape,

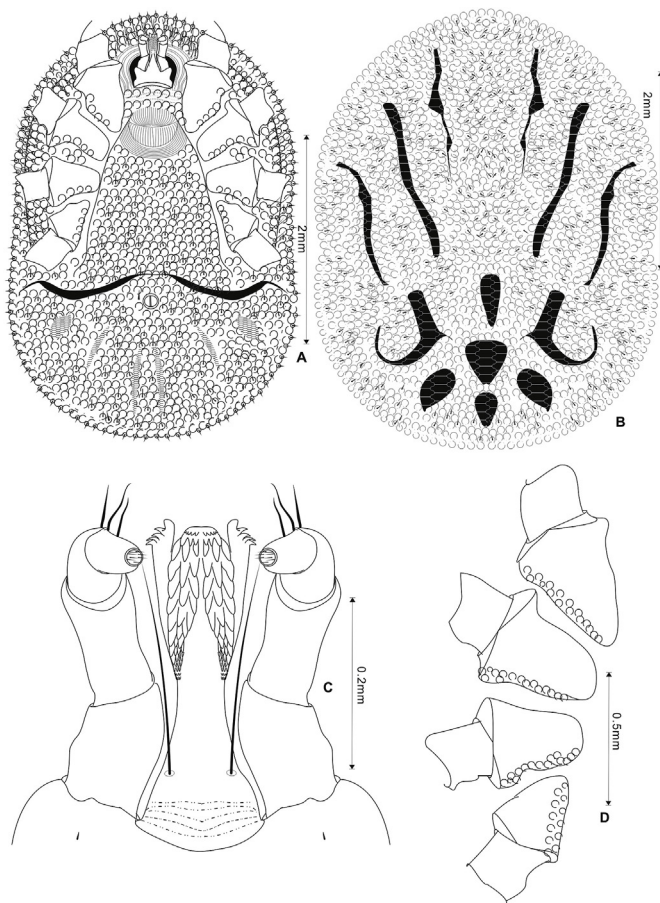


Fig. 1. *Ornithodoros huajianensis* n. sp. (female, holotype): A) dorsal view, entire; B) ventral view, entire; C) basis capitulum; D) Coxa I ~ IV.

somewhat raised, rounded apically; posterior mammillae moderate size, moderately spaced, bulbous shape, somewhat raised, rounded apically; all mammillae lacking buttresses, lateral surfaces relatively smooth (Supplemental Figs. S1 and A). A few of smaller mammillae with single seta mixed with larger ones (Supplemental Figs. S4 and B; Fig. 1, B). Discs conspicuous, slightly depressed, distribution and sizes as shown in Fig. 1. Under high SEM magnification disc showing some fine pebbling and narrow, incomplete furrows dividing surface into 8–10 unequal compartments. Anterior discs, 1 pair, thin-straited, short; anteromedian discs, 1 pair, stout straited, attaining the level of dorso-ventral groove; anterolateral discs, 1 pair, thin long straited, extending beyond the level of dorsoventral groove. The six discs distribute symmetrically to midfield axis, above the dorsoventral groove. Posterior to dorsoventral groove, 3 upside down sub-triangular discs situated on the midfield axis, the middle one widest; On each side of midfield axis, one inverted sickle shaped disc and one droplet disc arranged anteriorly and posteriorly respectively (Supplemental Figs. S1 and A; Fig. 1, B).

Venter Eyes absent; Margin thick and not clearly defined, integument leathery, distinctly mamillated as dorsal surface (Fig. 1, A; Supplemental Figs. S1 and B); Discs present only in grooves and depressions. Capitulum enveloped in a shallow camerostome without movable cheeks, lateral folds of camerostome distinct (Supplemental Figs. S2 and B; Fig. 1, A). There are numerous long and thick hairs above the capitulum, almost the anterior margin of the body, giving it a downy appearance (Supplemental Figs. S2 and B, Fig. 1, A). Supracoxal fold in coronal plane extending from anterior apex to slightly behind leg IV; ventral setae on anterior part of supracoxal fold long, numerous extending to trochanter III; setae on anterolateral hump of supracoxal fold long, slender, about twelve; setae on transversolateral part of supracoxal fold distinct, thick, blunt (Supplemental Figs. S2 and B).

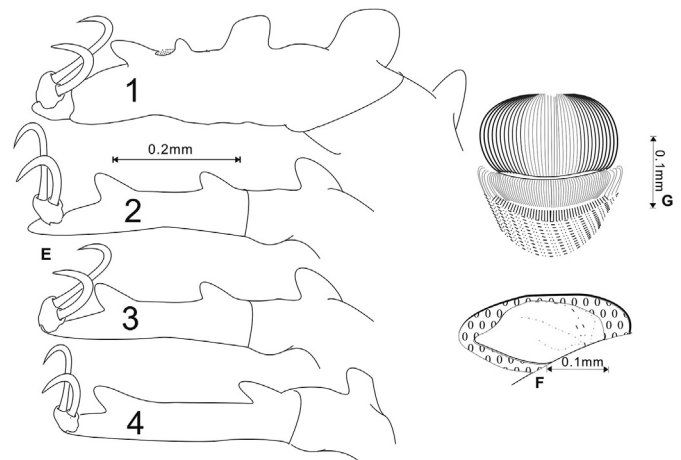


Fig. 2. *Ornithodoros huajianensis* n. sp. (female, holotype): E) Tarsi I ~ IV; F) Spiracle plate; G) genital apron.

Genital apron at the level of Coxa I, posterior to post-capitular area, somewhat raised; Anterior labium thin, transverse, with fine integumental ridges, numbered 40–45. Posterior labium thick, half-moon to gentle crescent shape, with relatively regular integumental ridges, numbered 50 (Supplemental Figs. S3 and A; Fig. 2, G). **Post-capitular area** wider than long, comprise of finely corrugated area proximally, through large mammillae centrally to flat, rough area distally at capitulum base (Supplemental Figs. 2 and A); **Post-genital area** with folded integument just posterior to genital apron, thin, with numerous mammillae, sub-oval, large (Supplemental Figs. S3 and A). **Dorsoventral groove** deep and broad, well-marked with closely spaced discs, extending laterally to body margin (Supplemental Fig. S1 B; Fig. 1, A). Anal rounded with a short quadratic depression behind it, 3 setae on each valve. Another 3 pairs of longitudinal furrows arranged symmetrically behind anal, with the central pair nearly paralleled. Both median postanal groove and transverse postanal groove are absent (Supplemental Fig. S1 B; Fig. 1, A). Spiracle plate ovoid, situated above the supracoxal fold, intermediated between leg III ~ IV, 0.288 ± 0.011 mm (0.276–0.299) in long diameter 0.138 ± 0.009 mm, (0.129–0.148) in short diameter. Each spiracle appears externally as slightly elevated convex box, the surface of which is divided by a deep, narrow and curving cleft into two structurally different portions. The anterior portion has a crescentic form and is perforated by innumerable minute pores which are distributed over its entire surface. The later portion (Macula) is formed of ordinary cuticle which is continuous with the general integument (Supplemental Figs. S4 and B; Fig. 2, F). **Capitulum** Basis capitulum (0.315×0.626 mm) situated in a camerostome folded by integument surroundings, base broad sub-rectangular, smooth, flat ventrally, sclerotized; anterolateral humps absent; post-hypostomal setae slender, 2/3 of hypostome length; post-palpal setae minute, stout, conical (Fig. 1, C; Supplemental Fig. S2, B), posterolateral setae numbered 5–7 pairs. **Palp** long, segment I (0.194×0.154 mm) surface partially finely pebbled, flange obsolete, not overlapping proximal part of hypostome; segments 2–4 (0.173×0.108 mm), (0.106×0.091 mm), (0.078×0.062 mm), smooth, breadth successively decreasing from 1 to 4; segmental length (1) 0.194, (2) 0.135, (3) 0.116, (4) 0.097, segmental formula 1.0 : 0.7 : 0.6 : 0.5. Dorsal setae on segment I short, blunt, peg-like, about four or five. Palpal segment II with four dorsomedial setae distally, three long, sub-central one slightly shorter; palpal segment III with two long dorsomedial setae (Fig. 1, C; Supplemental Figs. S2 and B). **Hypostome**, spatula-like, length from post-hypostomal hair to the corona 0.598 ± 0.004 (0.594–0.602) mm and 0.203 ± 0.008 (0.195–0.211) mm broad in base, ca. 3 times long as broad; moderately expanded sub-apically, truncate apically; denticles numerous, densely packed,

projecting laterally; large angular denticles about 40 in files of 3:3; chelicera apex robust, mobile bicusate tooth moderately large (Fig. 1, C; Supplemental Figs. S2 and B); *Coxae* elongate triangular, hemi-conical, anterior two-thirds sclerotized, posterior third as mammillated syncoxae, mammillae large (Fig. 1, D; Supplemental Figs. 1 and B); I, II somewhat swollen, II especially so; apex with margin raised, bearing constriction centrally; I, II, III, IV with setae on apical and subapical posterior margins, blunt, short; apical marginal hairs slightly longer on II, III, IV; on I about six, II about two apically only, III about four, IV about two. Trochanter simple with stout, blunt setae; I with setae on anterior margin four to five, two to three posteriorly; II four to five anteriorly, one to two posteriorly; III with three to four anteriorly, two to three posteriorly; IV with three to four anteriorly, one to two posteriorly (Supplemental Figs. 1 and B). Trochanterellus, about half length of the trochanter, setae on posterior aspect, short, sub-apical, one, occasionally two. *Legs*: moderate long, anterior slightly more robust than posterior; claws large without pulvillus. Femur twice length of trochanter, setae moderately abundant on anterior legs, sparse posteriorly; I, II, III, IV with setae on anterior and posterior margin, extending toward trochanterellus, I, II, III long, IV short. Tibia slightly shorter than trochanter; setae distinct, clubbed on apical margin. Metatarsus more than twice length of trochanter; dorsal lobes prominent, dorsoapical margin developed as additional lobe; setae on ventral margin arising from minor convexities. Tarsi narrow, gradually tapering distally, tarsus I mildly elevated dorsally, more than twice length of trochanter; three dorsal lobes prominent, equidistant; three lobes including a bipartite distal lobe where Haller's organ divides sub-lobes; proximal lobe tall, thick, rounded to curved; middle lobe long, moderated width, subrectangular to slight curved; distal sub-lobes well separated; proximodistal lobe wide, very short, distinct; distodistal lobe equilateral triangular to curved; setae on dorsal margin and ventral margin arising from minor convexities, mostly long, few short (Fig. 2, E; Supplemental Fig. 3 Panel B and C); Setae from lateral view on I–IV, respectively: apicodorsal 2, 1, 1, 1; subdorsal 8, 5, 5, 4–5; median 10, 9, 8, 10–11; apicoventral 2, 3, 3, 3; subventral 12–14, 7, 8–10, 10–11. Haller's organ roof flat, solid except for a narrow, slightly crescentic transverse aperture (through which 2 sensilla are visible), anterior pit setae about number 9 arranged as follows: 2 long serrate setae anterior to a group of shorter setae (1 conical, 2 porous, 2 fine, 2 grooved), posterior setae number 6 (4 short, 2 medium).

3.3. Male

Measurement and descriptions were conducted based on 3 males (Fig. 3; Supplemental Figs. S5–S6).

Body essentially as described for female, except for being slightly smaller, sized in 4.89 ± 0.68 (4.21–5.57) mm long and 4.69 ± 0.13 (4.56–4.72) mm broad (Fig. 3, B). Dorsum and venter as in female (Fig. 3, A, C, E), except the combination of the following characters. *Genital apron* surface smoother than surrounding integument, slightly posterior to the post-capitular area, somewhat raised. Anterior labium thick, transverse, with about 50 fine convoluted striates, tongue broad, sub-rectangular with slightly concaved anterior margin, Posterior labium short, broad triangular tongue-shaped (Fig. 3, F; Supplemental Fig. S6, Panel A). Tarsus I 0.489 ± 0.012 (0.477–0.501) long, with 3 prominent dorsal lobes as in female; tarsus IV: 0.62 ± 0.09 (0.53–0.71) long with dorsal lobes as in female. Tarsal setae from lateral view on I to IV, respectively: apicodorsal 3, 1, 1, 1; subdorsal 9–10, 4, 4, 3–4; medium 5, 7, 7, 7; apico-dorsal 2, 3, 3, 3; subventral 11–12, 7, 7, 9 (Fig. 3 D; Supplemental Fig. S5, Panel A–B).

3.4. Nymph

Measurement and descriptions were conducted based on 3 nymphs (Fig. 4; Supplemental Fig. S5.).

Body essentially as described for female, except for being much

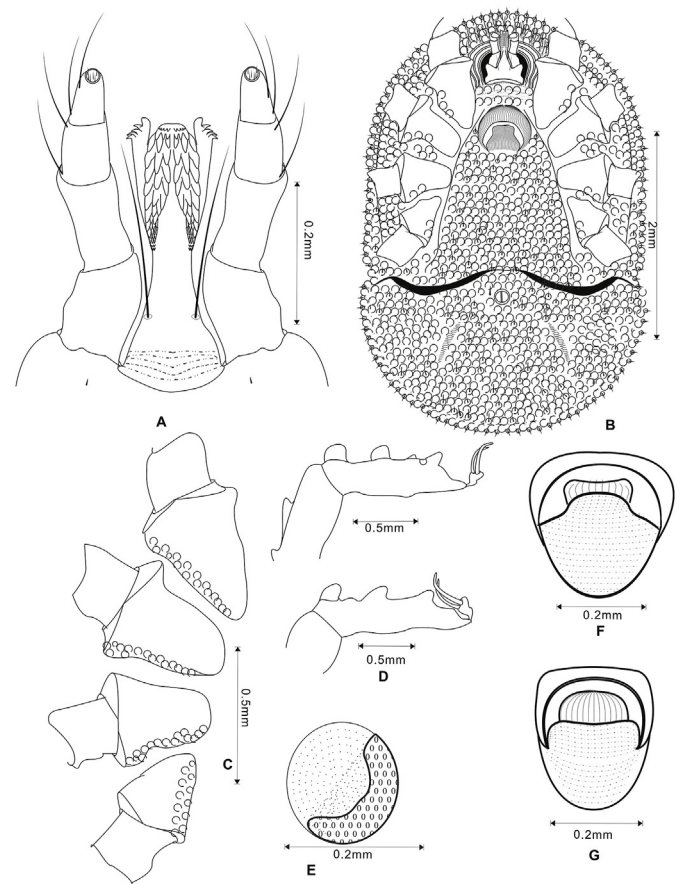


Fig. 3. *Ornithodoros huajianensis* n. sp. (male, paratype): A) basis capitulum; B) ventral view, entire; C) Coxae I–IV; D) tarsi II and IV; E) Spiracle plate; F) genital apron; G) comparative morphology of genital apron in *O. moubata*.

smaller, size in 4.59 ± 0.28 (4.29–4.81) mm long, 4.09 ± 0.17 (3.92–4.26) mm broad (Fig. 4, A). Dorsum and venter as in female (Fig. 4, B) except for absence of genital opening and the combination of following characters. Hypostome, spatula-like, length from post-hypostomal hair to the corona 0.542 ± 0.007 (0.535–0.549) mm and 0.184 ± 0.004 (0.18–0.188) mm broad in base, ca. 3 times long as broad; moderately expanded sub-apically, truncate apically; denticles numerous, densely packed, projecting laterally; large denticles angular, dental formula 2|2, in files of 7 or 8; small denticles about 20, scattered; chelicera apex robust, with mobile bicusate tooth moderately large (Fig. 4, C). Spiracle plate small, the maximal diameter is 0.18 ± 0.02 (0.16–0.20) mm (Fig. 4, E). Tarsus I 0.545 ± 0.06 (0.539–0.551) mm long, with 3 prominent dorsal lobes as in female, tarsus IV 0.59 ± 0.11 (0.48–0.70) mm long with dorsal lobes as in female. Tarsal setae from lateral view on I to IV (Fig. 4, D; Supplemental Fig. S5, C–D), respectively: apicodorsal 3, 1, 1, 1; subdorsal 9–10, 4, 4, 3–4; medium 5, 7, 7, 7; apico-dorsal 2, 3, 3, 3; subventral 11–12, 7, 7, 9.

4. Molecular observations

The sequences from male, female and nymph of *O. (O.) huajianensis* sp. nov. from the marmots in Huajian, Gulang county, Gansu province were identical when aligned using Clustal X and were deposited in the GenBank under accession no. MK208992.1 ~ MK208994.1. This finding of genetic identity confirms and conforms to the genetic associations between female, male and nymph of *O. (O.) huajianensis* sp. nov. from the same place. The phylogenetic relationships based on a partial sequence of the mitochondrial 16S rDNA gene grouped *O. (O.) huajianensis* sp. nov. with *O. (O.) compactus* and *O. (O.) moubata* within a

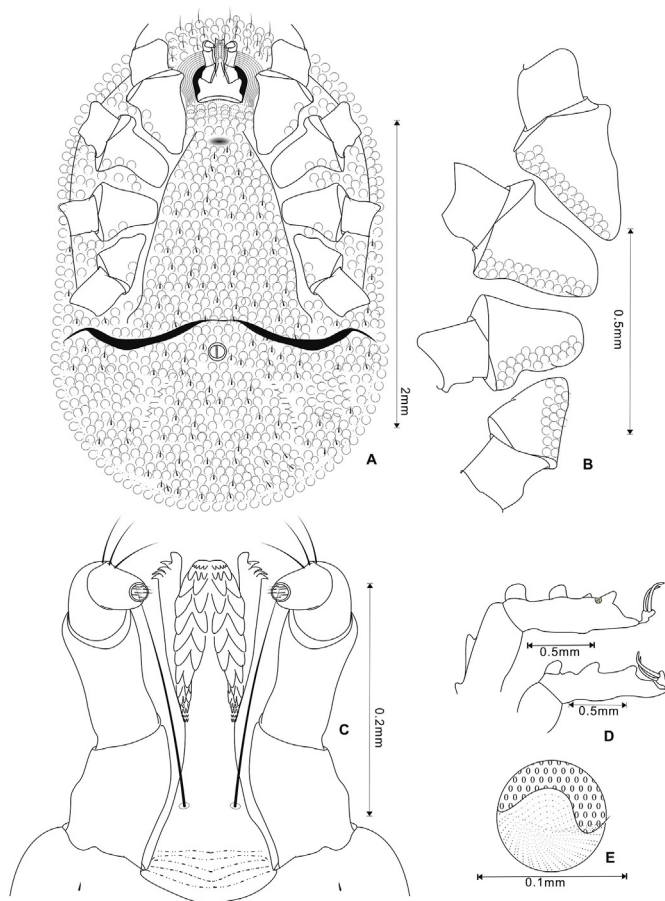


Fig. 4. *Ornithodoros huajianensis* n. sp. (nymph, paratype): A) ventral view, entire; B) Coxa I ~ IV; C) basis capitulum; D) tarsi II and IV; E) Spiracular plate.

strongly supported branch (88% bootstrap and 0.89 posterior probability) (Fig. 5). The 16S rRNA sequence divergence between *O. (O.) huajianensis* sp. nov. and *O. compactus* was 9.5%, between *O. (O.) huajianensis* n. sp. and *O. (O.) moubata*, 7.0% when 8.0% sequence divergence was presented between 16S rRNA of *O. (O.) compactus* and *O. (O.) moubata*, which meet the criteria of 5% or larger divergence between these species (Bakkes et al., 2018) and support the nomination of *O. (O.) huajianensis* as a new species in molecular phylogeny.

5. Taxonomic summary

Order: Ixodida Lech, 1815.

Family: Argasidae Koch, 1844

Genus: *Ornithodoros* Koch, 1844

Species: *Ornithodoros (Ornithodoros) huajianensis* Sun, Xu, Liu and Wu.

Type-host: *Marmota bobak sibirica* (Mammalia: Rodentia: Sciuromorpha).

Type-locality: Huajian village (N37°36'57.93 E103°04'24.91, elevation 1756 m), Gulang county, Gansu province, China.

Collectors and date of collection: Z. J. Liu, Jun 5, 1991. Type materials: **Holotype** female, was collected from female marmots collected in Huajian village, Gulang county, Gansu province, China, in 1991. It was preserved in alcoholic liquids deposited in the Medical Entomology Gallery of Academy of Military Medical Sciences, Beijing, China (AMMSC). (AMMSC-T-10823); **Paratype**, 3 nymphs, 2 females and 3 males, same data as holotype. Deposited in the Medical Entomology Gallery of Academy of Military Medical Sciences, Beijing, China (AMMSC). (AMMSC-T-10823 ~ AMMSC-T-10830).

Gene sequences: The mitochondrial 16S rDNA partial sequence of

Ornithodoros huajianensis sp. nov. generated in the present study was deposited in GenBank under the number MK208992.1 ~ MK208994.1.

Hosts and distribution: All females, males and nymphs of *O. (O.) huajianensis* were collected from the Mongolian marmots *Marmota bobak sibirica*. Since the tick species is only recorded in the marmots, the distribution of *Ornithodoros huajianensis* n. sp. is likely to follow the distribution of its marmot hosts, who prefer the semiarid hilly prairies as suitable habitation across China, Mongolia and Russia (Huang et al., 1995).

Etymology: The specific epithet derives from the original sites in allusion to the habitat where this species was found.

General: In accordance with section 8.5 of the International Code of Zoological Nomenclature (ICZN), details of the new species have been submitted to ZooBank with the life science identifier (LSID) zoo-bank.org/pub: 21FDEB36-F59C-436A-9473-BD415F358D52.

5.1. Remarks

The newly described species *O. (O.) huajianensis* was assigned into genus *Ornithodoros* for its distinctive morphologic features, including suboval flat body with blunt rounded anterior end, well developed denticulated hypostome and round margin not clearly defined (Nuttall et al., 1908; Clifford et al., 1964). Genus *Ornithodoros* is readily separated from genera *Chiropterargas* and *Carios* by the presence of paired organs or poorly developed transverse postanal groove, and from genus *Otobius* by the striated and spinose integument, panduriform body narrowed in middle (Clifford et al., 1964; Klompen and Oliver, 1993). Within the genus *Ornithodoros*, no *Microargas*, *Ornamentum* and *Ornithodoros* but for 2 *Pavlovskyella* species was recorded in China. The newly described species can be distinguished, in subgenus level, by the absence of cheeks around basis capitulum, transverse postanal groove and medium postanal one. The subgenus *Ornamentum* can also be differential diagnosed by distinctive ornated tops on the flatten mammillae (Clifford et al., 1964), whereas the unique features of the long, apical fringed setae around the transverse oval body outline (Hoogstraal and Kohls, 1966, 1973) can make the *Microargas* members be easily figured out. Considered that the presence of distinct dorso-ventral groove, preanal groove and the well-marked lobes on tarsi and metatarsi, together with the absence of eyes and lack of both transverse postanal groove and median postanal groove (Bakkes et al., 2018), we classified the newly described species *O. (O.) huajianensis* into *O. moubata* group of the subgenus *Ornithodoros*. The comparison of geometric morphologic shapes of tarsi I further indicates that the new species resembles with *O. (O.) indica*, *O. (O.) procaviae*, *O. (O.) eremicus*, *O. (O.) moubata* and *O. (O.) compactus* in *O. moubata* group. However, *O. (O.) indica* differ markedly with *O. (O.) huajianensis* by the dental formula 4/4 and 2 times longer palp II as palp I (Rau and Rao, 1971), although *O. (O.) indica* was the only species described from the foothills of Himalayas about 1500 Km away from Gulang county, China where the new species was found. Whereas, the presence of hood, triangular cheeks, 2 pairs of posthypostomal setae and much shorter palp II in *O. (O.) procaviae* from Israel in Palearctic realm (Theodor and Costa, 1960) separated it from *O. (O.) huajianensis*. And also, *O. (O.) eremicus* from North America in Nearctic realm, bears 2/2 dental formula on the hypostome, much shorter posthypostomal setae and the absence of lobe on tarsi V (Cooley and Kohls, 1941), which make *O. (O.) eremicus* distinguishable from *O. (O.) huajianensis*. Furthermore, the adults and nymph of *O. compactus* with flat and tile like mammillae integument, shows distinctive morphological differences from *O. (O.) huajianensis* n. sp. and *O. (O.) moubata*, whose mammillae are bulbous, sub-equally sized and tightly spaced (Bakkes et al., 2018). *O. (O.) huajianensis* n. sp. closely resembles *O. (O.) moubata*, from which, however, it may easily be distinguished by the details of male genital apron (Fig. 3, F), the presence of a shallow camerostome and smaller mammillae with single seta mixed with larger ones (Supplemental Figs. S2 and A; Supplemental Figs. S4 and A). Whereas the broad crescent

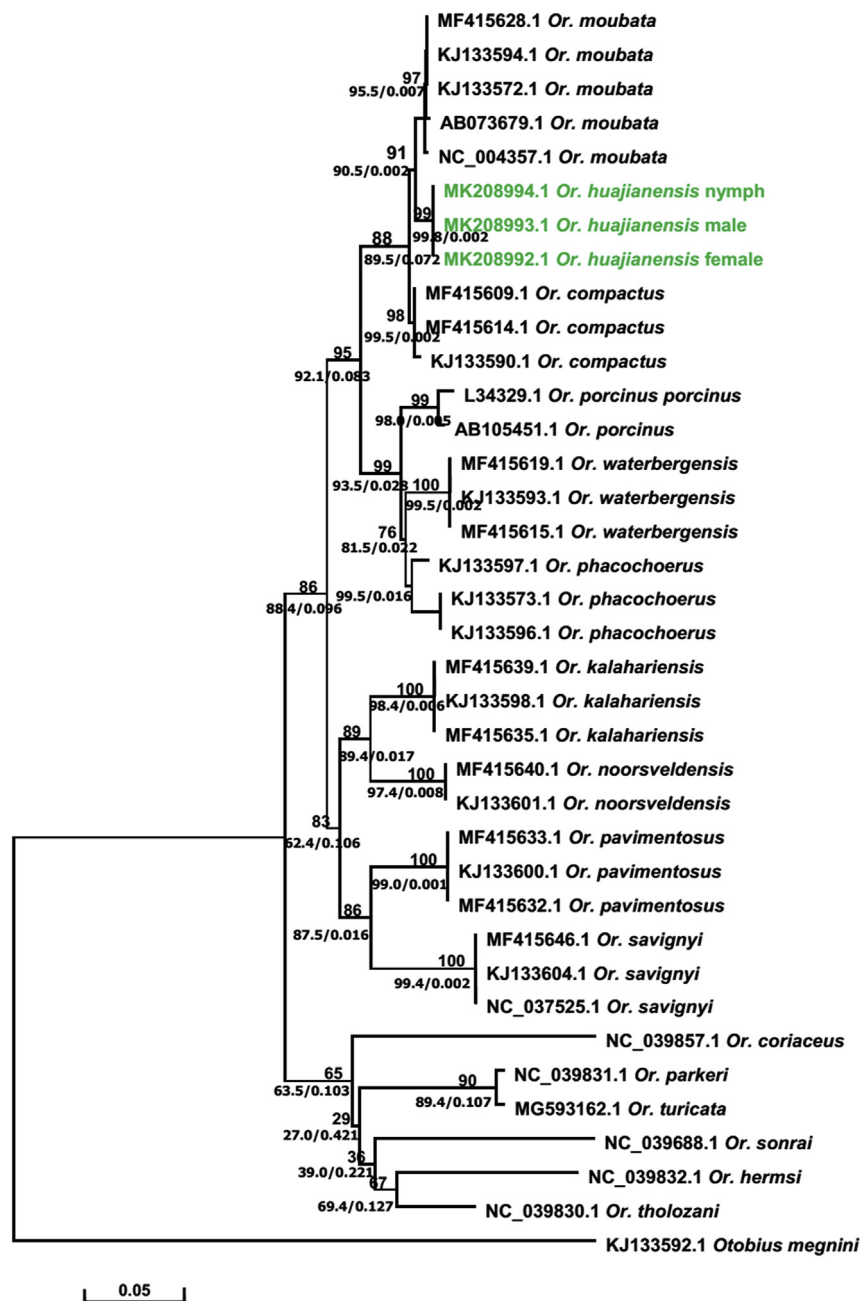


Fig. 5. Phylogenetic tree including *Ornithodoros huajianensis* n. sp. and other selected species of *Ixodes* based on 16S rDNA. The alignment was produced using Clustal X and the tree was inferred by means of the MP method with 500 replicates of random addition. The species *Otobius megnini* was used as outgroup. The Bayesian support (posterior probability) values are derived from 1,000,000 replicates.

tongue and short, broad sub-rectangular posterior labium with slight concaved anterior margin presents in the male genital apron, no camerostome or smaller mammillae visible in both sexes of *O. (O.) moubata* (Fig. 3 G, Supplemental Figs. S6 and B).

Data from 16S rRNA genes also agree to distinguish *O. (O.) huajianensis* n. sp. and test the monophyly of the subgenus *Ornithodoros* in the phylogenetic analysis as shown in Fig. 5. There are two clades formed due to the genetics difference between the *O. (O.) moubata* group and *O. (O.) savignyi* group, which is supported by the absence or presence of eyes in phenotypic morphology. The new species is closely related to *O. (O.) moubata* (Bayesian posterior probabilities 0.905), occurring in South Africa, Zimbabwe (unconfirmed records in Mozambique, Tanzania, Kenya and Botswana) (Nuttall et al., 1908; Bakkes et al., 2018) and *O. (O.) compactus* (Bayesian posterior probabilities

0.895) restricted to South Africa (Walton, 1962). The former species was recorded parasites on warthog, porcupine, pangolin, pig, goat, dog and occasionally human, while the later species were only found on tortoise including *Chersina* spp., *Geochelone* spp. *Homopus* spp. *Psammobates* spp. and *Testudo* spp. Therefore, a new species is nominated as *O. (O.) huajianensis* strongly supported by morphologic and phylogenetic evidences.

6. Discussion

Argasidae taxonomy advanced substantially over past decades with the refinement of morphological description and the increased utilizations of genetic analyses. Great diversity of the family Aragsidea had been well presented along with the recent upsurge in the discovery of

argasid species, with 26 new species described, resurrected or unique sequences published (Bakkes et al., 2018; Barros-Battesti et al., 2015; Burger et al., 2014; Dantas-Torres et al., 2009; Dupraz et al., 2016; Heath, 2012; Labruna et al., 2016, 2011; Muñoz-Leal et al., 2018, 2017, 2016, 2008 Nava et al., 2013, 2010; Sangioni et al., 2008; Venzal et al., 2015, 2013, 2012, 2008; Vial and Camicas, 2009). Along with this progress, gaps on argasid systematics among different classification schemes come to be bridged gradually for the pronounced cladistic analysis based on 83 morphological features (Klompen and Oliver, 1993) and the reliable phylogenetic studies on the nuclear and mitochondrial sequences of the family Argasid (Burger et al., 2014; Dantas-Torres, 2018; Beati and Klompen, 2019). Following the monophyletic theory of biological taxa, a revised argasid list was proposed to resolve the systematic issues on the paraphyly of the expired genera of Argas and Ornithodoros based on the evolutionary context retrieved from mitochondrial genome (Mans et al., 2019). Under the newly proposed classification scheme, the name-bearing type genus Ornithodoros involve 47 valid species within four subgenera, namely Ornithodoros, Ornamentum, Microargas and Pavlovskyella when previous Theriodoros was treated as a synonymize of Pavlovskyella as the minor differences in species level appeared inadequate to make diagnosis between subgenera Pavlovskyella and Theriodoros (Clifford et al., 1964). Among them, subgenus Ornithodoros had been expanded to 13 species and divided into two groups with the integrative taxonomic evidences from geometric morphology, bioecology and related mitochondrial phylogeny (Bakkes et al., 2018). The *O. moubata* group was known to embrace 9 non-eyed species, while the rest 4 eyed ones were placed in *O. savignyi* group. The newly described species *O. (O.) huajianensis* was classified into *O. moubata* group with diagnosis characters synthesized from female, male and nymph samples as follows. (1) Discs arranged in grooves or depressions. (2) The absence of transverse postanal groove, ventral plates and cheeks, (3) The pronounced preanal groove, dorsal lobes on tarsi and metatarsi (Clifford et al., 1964). However, the broad rectangular tongue and broad triangular tongue-shaped posterior lip in male genital apron, a shallow camerostome with definite folds and smaller mammillae with single seta mixed with larger ones in nymph and adults observed in the new species made itself very unique and easily separated from its congeners in the subgenus Ornithodoros. The phylogenetic evidences derived from 16S rRNA sequences also support the assignment of *O. (O.) huajianensis* as a new member of the subgenus Ornithodoros. Since nymphs and adults of most argasid species are often inadequate for taxonomy due to the lack of external characters suitable for species identification, quite a few of high rank taxon of argasidae were classified by the diagnosis characters derived from larvae (Sonenshine et al., 1962; Klompen, 1992). Unfortunately, we failed to obtain larval specimens of *O. (O.) huajianensis* sp. n. and judge its nymph instar of the specimens, which inhibited our availability to make thoroughly comparisons between similar species morphologically.

Among the 13 valid species in the subgenus Ornithodoros, only 3 species were documented outside the Afrotropical realm, including *O. (O.) indica* from Udaiguri in India of the Indomalayan (Oriental) realm, *O. (O.) procaviae* from Lahavoth haBashan in Israel of the Palearctic realm and *O. (O.) eremicus* from Utah in United States of the Nearctic realm. The disparity of distribution patterns of subgenus Ornithodoros might attribute to the 'Africa-first' hypothesis on the Gondwana origins of ticks and their possible migrant route from African to Eurasia driven by vicariance, habitat conditions as well as the possible introductions of wild boars or other associated hosts (Beati and Klompen, 2019). The discovery of *O. (O.) huajianensis* sp. n. in Gulang, Gansu province would enrich Chinese Argasidea fauna by adding a new record subgenus with one new species. According to the latest checklist for Argasidae (Mans et al., 2019), there would be two valid subgenera Pavlovskyella and Ornithodoros of genus Ornithodoros in China. Moreover, the increasing valid Ornithodoros species outside the Afrotropical realm would remarkably improve the phylogeographic researches on the

subgenus Ornithodoros, although their distributions would range much wider than our knowledges. Historically, the term 'Ornithodoros' was derived from Greek word 'Ornis' which means bird, however, only one species *O. (P.) macmillani* was recorded from cockatoo in Austrasia regions, most species in genus Ornithodoros and all species in the name-bearing type subgenus parasite on the hosts ranged from reptiles to mammals, including rodents, warthogs, porcupine and deer (Mans et al., 2019). Since tick-host relationships were considered to be mostly species specific, the phylogeographic history of Ornithodoros ticks and their radiation patterns of their main lineages might also be mirrored by hosts evolution (Hoogstraal, 1985; Beati and Klompen, 2019). Considered that the herbivore Mongolian marmots (*Marmota bobak sibirica*) usually live in the burrows ca. 2 m beneath the earth, the new species *O. (O.) huajianensis* would survive the endophilic habitats with high suitability, as other burrowing Ornithodoros species do. The possible immigrant of the new species from places out of Eurasia should be expelled out because the suitable habitats for the marmots evolved to adapt are strictly limited and no transportation of marmot occurs within Chinese territory. Although we are still unable to define the accurate host spectrum and the human infestation capability of *O. (O.) huajianensis* based on current available data, its affinity relationship with *O. (O.) moubata* would also suggest the immense medical significance of *O. (O.) huajianensis* as potential vectors to transmit African swine fever and other Ornithodoros-borne diseases, which should be determined in the coming future.

7. Conclusions

The morphological and phylogenetic evidences from the *Ornithodoros* adults and nymph collected from *Marmota bobak sibirica* made it possible to describe a new species named *Ornithodoros huajianensis*. This is the first report of argasid ticks on Marmots in China, which expands the number of species of the genus *Ornithodoros* for China.

Declarations

Ethics approval and consent to participate

The animals were caught and manipulated in accordance with the recommendations of the Institutional Animal Care and Use Committee of Institute of Zoology, Chinese Academy of Sciences. (IACUC-IOZ). The aim of the design and detail protocols were informed and consent to all participates.

Availability of data and materials

The data supporting the conclusions in this study are included in the article.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare that they have no competing interests.

Authors' contributions

Y. Sun and RM Xu conceived the study and described the new species; Liu ZJ collected the ticks from the hosts and prepared the specimens for morphological studies; MY Wu and T. Qin carried out the molecular genetic studies and performed the sequence alignment and phylogenetic analyses; Y. Sun drafted the manuscript; RM Xu reviewed it critically for important scientific and intellectual content; and all the

authors read, reviewed and approved the submitted version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpaw.2019.05.001>.

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