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Morphological and phylogenetic tree analysis of *Argas persicus* in local chicken in Mosul city

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Abstract

The current study meticulously classified soft ticks, *Argas persicus*, according to the taxonomic dichotomous key, which consists of many parameters. The ticks showed a mamillated, granulated, written, tinkled, or striate structure with the advantage of having a pair of claws on each tarsus, which were carefully observed and documented in our research. In *Argas persicus*, the separation between males and females was the genital cleft, which is a broad horizontal fissure in the females, while in males, have halved width of females and is more ovoidal in outline. Haller's organ is also a complicated sensory organ enchanted by *A. persicus* and has a chemosensation instead of insect antennae. Seven hundred sixty-four specimens of soft ticks collected as soon as they dropped off the bird have been seen both for individual ticks and for batches. The specimens of the ticks were identified to species level under a stereoscope dissecting microscope based on the morphological characters. *A. persicus* collected from local chicken and their shelters from many regions of Mosul city (north, east, west, and south of the city). The polymerase chain reaction for the COX1 mitochondrial gene had been positive in all samples; the purified amplicons had been sent to a commercial company for sequencing. The present study focused on the morphological characteristic of *A. persicus* to achieve guideline witness with photos taken under stereoscopic dissecting microscope, which was established by the phylogenetic analysis of four isolates of *A. persicus* OR995800, OR995801, OR995802, and OR995803. The convergence ratio between our isolates and those of China, Iran, Pakistan, South Africa, USA, Duhok/Iraq, and Italy was 95.36-100%, and the phylogenetic tree showed that the four isolates fell within one clade.

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Introduction

A Wide Geographic Distribution of *Argas persicus* inhibits disparate Climate Areas worldwide (1), especially in poultry which have an important role for meat food sources in food industry (2,3) and thus diverting the observation of legislators of many countries populations from the demand for acceptable and equitable development, assembled on many districts of economic (4) and classification of the evaluated effectiveness of composite films of *A. persicus* toxins on the chemical qualities of chicken meat during storage at 4°C for many days (5). The morphological features of *Argas persicus* have been identified in northern parts of Africa, Such as Tunisia, Libya, Algeria, and Egypt (6). The owl tick or Miana bug is the common name of *Argas persicus.* The dorsal surface of this tick sieveplate shows the airplanes, especially during molting. The covering of the new spiracle is made around the ecdysial spiracle with the sieveplate, baseplate, and labyrinth formed as one unit (spiracular plate) and the spot as another, just as Ixodidae, so this process makes the fowl tick adapted to inhibit different climate areas (7,8). There are incompetent taxonomic descriptions and incorrect assignments for some species of tick, which leads to getting adequate knowledge about the

natural history of ticks and dispersal with their ability to transmit bacterial and viral agents to the hosts (9). *A.* p *rescue* is well thought-out endemic to the areas of central Asia, as arborous nesting birds (10). Argas (Persicargas) group consists of 16 ornithophilous species phenotypically similar (11). Awwad *et al.* (12) investigated the molecular identification of three species of Argas by using polymerase chain reaction (PCR), showing that Argas species may be polyphyletic. The morphological characteristics of the tick represented as bird-feeding ectoparasite were clarified in 1827 by Audouin from Egyptian samples. He explained that *Argas hermanni* is similar to *Argas reflexus* and *A. persicus* in the architecture of their bodies (13,14).

The study proved how the tree could support some of the already proposed systematic adjustments based on morphology alone. It showed no main differences with other previously announced trees using either mitochondrial or nuclear, coding or non-coding genes.

Materials and methods

Ethical approval

The institutional animal care and committee in the College of Veterinary Medicine, University of Mosul, ethically permitted this study numbered UM.VET.2023.093 dated on 22/6/2023.

Tick collection

Opportunistically, ticks had been collected from poultry farming cages in many regions of Mosul (North: Al-Sada and Baawyza, Al-Arabi, and Al-Rashydia). East: Al-Khathraa flats, Al-Karamah, Kokjali, Al-Qadysia and Al-Zahraa. West: Bab Al-Jadeed, Tal Al-Ruman, Al-Yarmuk and Hawii Al-Kaniisa. South: Al-Wahdaa, Somar, Yaremjaa) between June and October 2023. Seven hundred sixty-four specimens of soft ticks collected as soon as they fell off the bird have been seen both for individual and batches of ticks (15). The collected ticks were preserved in absolute ethanol until laboratory analysis. Initially, ticks were morphologically detected following Estrada-Peña *et al*. (16) and Monti *et al*. (17). The specimens of ticks were identified to species level under a stereoscope with 25X magnification power based on the morphological characters constructed by Kohls *et al.* (18). To analyze and prepare the ticks, images of body parts. The taxonomic dichotomous key of *Argas persicus* has been summarized by the following characteristics: the body of *A. persicus* is flattened dorsoventrally, and the margin of the body makes a lateral suture. The structure of this suture consists of rectangle-like plates, both at the dorsal and ventral aspect. The integumental structure is complicated, and there is also no presence of eyes; there is a pair of claws on each tarsus. The mouthparts are small and ventral. They are composed of a central toothed hypostome with a pair of palps. Well, the genital cleft is a broad horizontal fissure in females, while males have a genital cleft in the exact location; although it is approximately half the width of that of the female and more oval in outline, the stigma shape is a cone situated above the coxae of legs 3 and 4. Hall's organ is a complicated sensory organ possessed by soft ticks, and it has a chemosensation instead of insect antennae but is structurally different. Generally, the *Argas persicus* has been described as mamillated, granulate, wrinkled, or striate. Dors l shield or scutum is absent in the post-larval instars of nearly all family members Argasidae, which is treated as a modern or derived character (15-19). Also, the total body shape was captured using a digital camera with sensor-shift Ois and autofocus for closeup without losing quality.

Phylogenetic analysis

After DNA extraction and selection of relevant primers (20-23), the polymerase chain reaction for the COX1 mitochondrial gene had been positive in all samples; the purified PCR amplicons were sent to a commercial company for sequencing (Macrogen, Inc., Seoul, Republic of Korea) (20-21). PCR has been done by using specific primers to confirm the molecular detection of all accrued *A. persicus* isolates *16SrRNA*-F, *16SrRNA*-R targeting the *16SrRNA* gene, also COX1-F and COX1-R harassing COX1 gene (Table 1). The phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura- Nei model in MEGA11 software and bootstrap analysis with 100 resampling. Partial DNA sequences of concatenated partial COX1 gene were used as input data (22).

Table 1. Sequences of primers used for amplification of specific genes of *Argas persicus*

Primer	Primer Sequence 5' – 3'	Product size (bp)	References
16SrRNA-F	TTTGGGACAAGAAGACCCTATGAATTT	240	(20)
16SrRNA-R	ACATCGAGGTCGCAATCAATTTTATC		
COX1-F	GGAGGATTTGGAAATTGATTAGTTCC	606	(21)
COX1-R	ACTGTAAATATATGATGAGCTCA		

Results

Morphological features

Figure 1 shows the tick's body. This figure also clarifies the mamillated, granulate, wrinkled, or striate structure of *Argas persicus.* The results of this research were shown by relying on morphological description: The adult females had a bluish-grey coloration, while those not engorged with blood looked like brown shells. *Argas persicus* has the advantage of having a pair of claws on each tarsus (Figure

2). The mouthparts are small and ventral; they consist of a central toothed hypostome combined with a pair of palps; the shape of the Stigma is a cone, which is situated superior to the coxae of legs 3 and 4 (Figures 3). One of the essential features of the *Argas persicus* that disperses between males and females is the genital cleft, a broad horizontal fissure in the females. However, males have a halved width compared to females and are more ovoid in outline (Figure 4). Haller's organ is a complicated sensory organ enchanted by *A. persicus*. It has a chemosensation instead of the antennae of an insect but is structurally different (Figure 5).

Figure 1: (A) Body of *Argas persicus* is flattened dorsoventrally. (B) The mamillated, granulated structure is obvious.

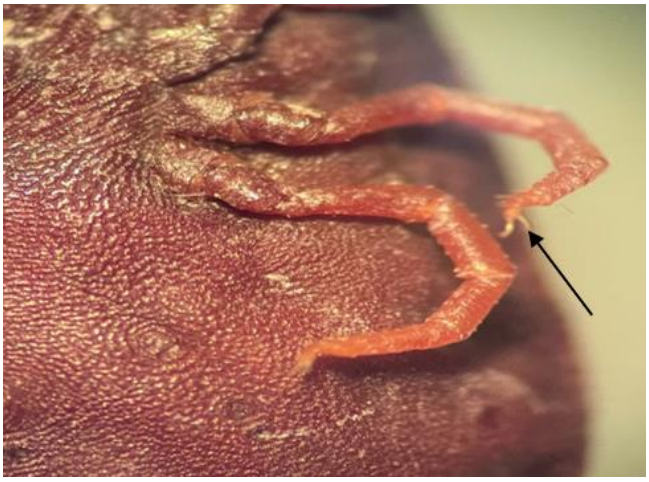

Figure 2: A pair of claws on each tarsus of *Argas persicus* leg (black arrow).

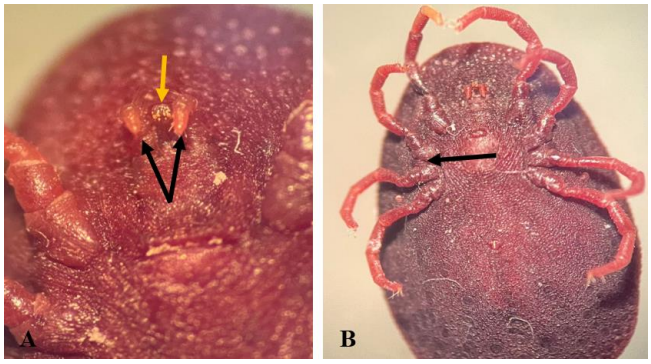

Figure 3: (A) Central toothed hypostome (yellow arrow) with a pair of palps (black arrow). (B) Stigma, a conelike shape of *Argas persicus* (black arrow).

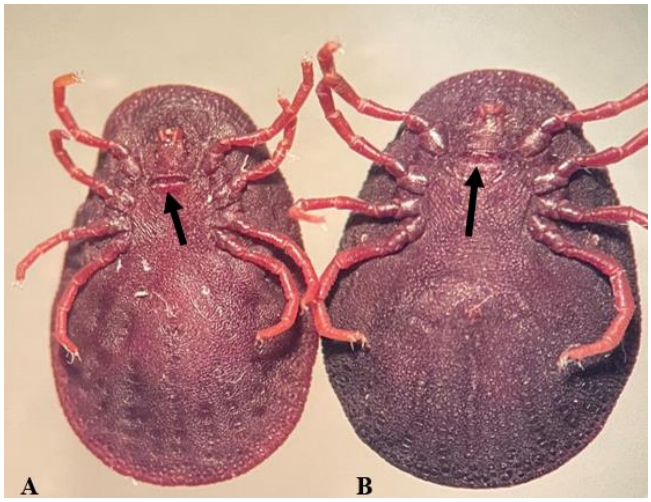

Figure 4: (A) Genital cleft of *A. persicus* male (Black arrow). (B) Genital cleft of *the female Argas persicus* (Black arrow).

Figure 5: Haller's organ is a complicated sensory organ possessed by soft tick *A. persicus* (Black arrow).

Phylogenetic analysis

Blast comparisons proved the morphological detection since both COX1 genes showed (95.36%-100%) identity with analogous sequences of the soft tick *Argas persicus* from other countries. The analysis of the phylogenetic tree clarified the existence of gene sequencing of sixteen samples that had been sent to Microgen company in South Korea and diagnosed in Mosul city for *A. persicus*. They were recorded in NCBI to get the accession number for the local sequencing gene of *A. persicus*, as shown in table 2. 75% bootstrap support). The results of the BLAST of the captured COX1 sequences of soft ticks had been showing 95.36%-100% integrity with *A. persicus* from China-LC209196.1, Italy-PP259541.1, Duhok, Iraq-MK287892.1, China-MK571448.1, China-FN394341.1, China-MN900726.1, Iran-KX879770.1, Pakistan-OQ860245.1, South Africa-OP326585.1, USA-ON800828.1, Pakistan-MW077849.1, China-OM368319.1, and South Africa-KJ133581.2. In the phylogenetic tree, the obtained sequences were clustered with revealed sequences (Figure 6). The consensus sequences of *A. persicus* were uploaded to GenBank (OR995800, OR995801, OR995802, and OR995803). The phylogenetic tree appeared typical of *A. persicus* grouping in one clade, which assisted with high bootstrap analysis.

Table 2: Sequence identity between local *Argas persicus* tick species and others have been recorded in the GeneBank for COX1

No.	*Argas persicus* tick species	GenBank accession number	Country	Identity (%)
1	*Argas persicus* isolate GY-AP5	MN900726.1	China	100
2	*Argas persicus* isolate 95-6-13	KX879770.1	Iran	100
3	*Argas persicus* isolate AP1	OQ860245.1	Pakistan	100
4	*Argas persicus* isolate 43	OP326585.1	South Africa	100
5	*Argas persicus*	ON800828.1	USA	100
6	*Argas persicus* isolate H1	MW077849.1	Pakistan	100
7	*Argas persicus* isolate E5	OM368319.1	China	100
8	*Argas persicus* clone Contig35_14411_396	KJ133581.2	South Africa	100
9	*Argas persicus* strain GS	MK571448.1	China	99.77
10	*Argas persicus* isolate ASS	FN394341.1	China	99.77
11	*Argas persicus*	MK287892.1	Duhok, Iraq	99.64
12	*Argas persicus* isolate AP-Z752-2022	PP259541.1	Italy	99.3
13	*Argas persicus* isolate: ALX	LC209196.1	China	95.36

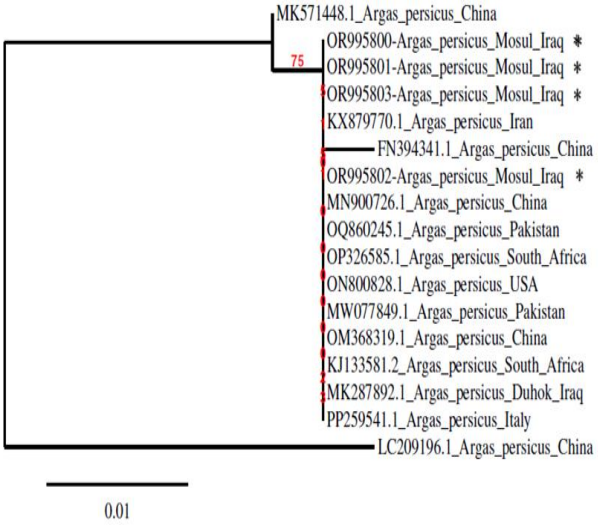

Figure 6: Phylogenetic tree of *A. persicus* Iraq (*). The phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEG11 software, and bootstrap analysis with 100 re-sampling partial DNA sequences of concatenated partial COX1 gene were used as input data and bootstrap support values.

Discussion

Many researchers are investigation to understanding the phylogenetic analysis of many pathogenic agents transmitted by hard and soft ticks, like Theileria species, *Anaplasma phagocytophilum*, *Babesia caballi* to the animals in Mosul city I want, as well as those researches which deal with the health status of birds in Nineveh province and Iraq in general, moreover the investigation of the effect of Ivermectin in chicks and those studies that dealt with the commonest diseases of chicken and their influences on health in general of the chicken, also expanding of the studies that touched upon the arthropods and their effects on animals (21-31). This brought us together to focus on arthropods, specifically soft tick *Argas persicus*, for the first time in Mosul city and local chicken at the morphological and molecular analysis level.

The present study reported morphological identification of *A. persicus* collected from chickens from different Mosul city areas. *A. persicus* spread widely worldwide, habituated to distinct climatic conditions. The morphology of *A. persicus* tick was observed using a stereomicroscope or dissecting stereomicroscope (32). morphological analysis in the present had been matched with the tick morphology of the previous studies. However, some differences have been

observed; the interspecific changeability in the phenotype of the ticks may be demonstrated either by the lower number of measured specimens or by the different geographical origins of the analyzed material, for instance, tick samples measured by Kohls *et al.* (18) had been obtained from females and late stage of nymphs in different avian hosts from USA.

The segregation in morphology between *A. persicus* in the present study and other species of Argas in several countries showed regular lateral integumental cells, or what had been called rectangular plates, as they appeared in this work, and irregular lateral plates of Argas spp. in Egypt (14). The climatic gradient could affect the shape of integumental cells and the size of these cells of *A. persicus* according to the ecogeographical adaptation evolution rule, that *A. persicus* has small and circular integumental cells in a cold climate in d Central European Countries, Czechoslovakia, Dusbabek (33).

Also, the small Stigma or spiracular plates are slight, and the eyes are absent, as it had been showing by Kohls *et al.* (18) and had been indicated that the Stigma of Egyptian *A. persicus* situated lateral to Coxa IV and the genital cleft was a transverse aperture situated between Coxae I, Kohls, *et al.* (18) mentioned that the tarsi of the legs might be so nearly elevated, but not subapically humped as it had been found in the current study (Haller's organ), the difference is attributed to the ecogeographic role in biological adaptation for climatic gradient (34,35). Our results agreed with Walker *et al.* (1) that the proximal region of the legs' tibia has two claws in the *A. persicus* collected from different areas of Africa.

The systematic track records of the family Argasidae go back to the beginning of the 20th century. However, the first theories about phylogenetic affinities among members of this family were proposed by Pospelova-Shtrom (36) and Klompen (37), who carried out the first phylogenetic study of Argasidae using a cladistic methodology based on shared derived characters to infer evolutionary history. Sequencing and phylogenetic analysis of two mitochondrial genetic markers confirmed the morphological results concerning *A. persicus* (38). Phylogenetic analysis of COX1 sequences of *A. persicus* involved 23 nucleotide sequences: South Africa KJ133581.2, Pakistan MW077849.1, and China MN900726.1, as a result of Gallab *et al.* (14), who agreed with our outcomes, especially for South Africa and China.

The study was conducted in Iran to sequence the COX1 gene collected from *A. persicus*. Yavari *et al.* (21) showed that there was no agreement with our study, particularly with the sequences of South Africa GU355920.1 and China KR297209.1. The discrepancy appeared because of the periphery of *the A. persicus* population, the animal husbandry farmers' movement, and the exportation of birds from country to country (39). The phylogenetic relationship of *A. persicus* stems from different areas around the world. As etimated by Rahmani *et al*. (39), most Algerian samples showed adjacent phylogenetic alliance together, possibly related to the same sampling area of soft ticks where associated tick generation has evolved with multiple life cycles in the hosts and the same environmental condition, so the convergence ratio between our isolates and those of the other countries China, Iran, Pakistan, South Africa and USA had been 100% which was very close to the result of Muñoz-Leal *et al.* (9) 99-100%. For that mitochondrial 16SrRNA sequence to *A. persicus* from Brazil and Chile and this explains the great convergence between our result with Muñoz-Leal *et al.* (9) despite of the vastly distended geographical distribution, the reason may be attributed to the same evolutionary history of these species (40). Phylogenetic detection of fowl tick *A. persicus* in our inquiry showed the closet resemblance of more than 92% to the sequences of GenBank conveyed from assorted areas of the world included China, Italy, and Duhok/Iraq (23,41,42)

Conclusion

The phylogenetic tree analysis of the soft tick on chicken *Argas persicus* from the four sides of the city was recorded for the first time in the current inquiry in the city of Mosul. There is 95.36- 100% identity between incidents of *A. persicus* both in Mosul and six countries included China, Italy, Duhok/Iraq, Iran, Pakistan, South Africa, and USA had been confirmed. Comprehensive, full-scale research is desirable in the coming years for greater clarity of ecology and *A. persicus* distribution in the different meteorological areas of Nineveh province, as well as its impact on the production of poultry and industry chicken farms and the role of the morphological study of these populations of soft tick in approves different relations, which probably signify distinct species.

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Conflict of interest

The authors declare that there are no contests of interest about the publication of this article.

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التحليل المظهري وشجرة النشوء والتطور للبرام الفارسي في الدجاج المحلي في مدينه الموصل

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الخلاصة

وضحت هذه الدراسة أن القراد اللين يمكن تصنيفه بالاعتماد على مفتاح التصنيف ثنائي التفرع والمؤلف من عدة معايير، عشرة منها أخذت بنظر الاعتبار في بحثنا هذا للتعرف على البُرامُ الفارسي، إذ ظهر جسم القراد بمظهر حبيبي مجعد أو مخطط مع تميزه بامتلاك زوج من المخالب على كل رسغ ،أجزاء الفم صغيرة وبطنية الموقع مع وجود منطقة تحت الفم المسننة والمركزية الموقع والمتحدة مع زوج من اللوامس فضلا عن الفتحة التنفسية مخروطية الشكل والواقعة اعلى حرقفتي الرجلين الثالثة والرابعة كما قد تمت الإشارة اليها .في قراد البرام الفارسي كان التمييز بين الذكور والإناث من خلال الشق التناسلي الذي يكون عريض في الإناث وبنصف العرض في الذكور مع كونه اكثر بيضاوية فيها، كذلك عضو هالر وهو عضو حسي معقد ذو تحسس كيميائي كبديل لقرون الاستشعار في الحشرة. ٧٦٤ عينة من القراد اللين حال سقوطها من على الطائر أما بشكل قرادات منفردة أو دفعات من القراد اللين والمجموع من الدجاج المحلي ومأوى هذا الدجاج من المناطق المختلفة في مدينة الموصل (شمالا وشرقا وغربا وجنوبا للمدينة). تم إجراء تقنية تفاعل السلسلة المتبلمر للجين 16srRNA والجين mitochondrial COX1 والذي كانت نتائجه موجبة لكل العينات وان الأجزاء الناتجة من مضاعفة الحمض النووي. ركزت الدراسة على الخصائص الشكلية البُرامُ الفارسي لتحقيق الدليل الشاهد مدعوماً بالصور المأخوذة البُرامُ تحت المجهر التشريحي المجسم والذي تم ترسيخه من خلال تحليل النشوء والتطور لأربع من عزلات البرام الفارسي OR995802, OR995801, OR995800, OR995803، وكانت نسبة التقارب بين عزلاتنا وتلك من الصين، إيران، باكستان، جنوب أفريقيا، الولايات المتحدة الأمريكية، دهوك/العراق وإيطاليا كانت ٩٥,٣٦ -١٠٠% مطابقة، وأظهرت الشجرة الجينية بأن العزلات الأربعة واقعة ضمن فرع حيوي واحد.