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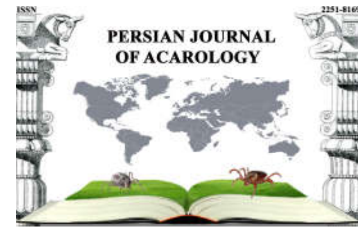

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Aegyptianella pullorum (Rickettsiales: Anaplasmataceae) in tick *Argas persicus* (Acari: Argasidae) from Iran: a preliminary assessment

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Aegyptianella pullorum (Rickettsiales: Anaplasmataceae) is an obligate intracellular organism which is most closely related to *Anaplasma* spp. (Abdul-Aziz and Barnes 2013). *Aegyptianella pullorum* is the causative agent of avian aegyptianellosis, a noncontagious infectious disease of chickens, geese, ducks, quail and ostriches, and is transmitted by argasid ticks as biological vectors (Kreier and Gothe 1976). Although, *Ae. ranarum* is transmitted by the amphibian-feeding leech *Batracobdella picta* (Zhang and Rikihisa 2004). Nine species have been assigned to the genus *Aegyptianella* (Gothe 1992). The best known, most widely occurring species and representative of this avian infecting microorganism is *Ae. pullorum* (Glomski and Pica 2016). Both species of *Aegyptianella* (*Ae. pullorum* and *Ae. moshkovskii*) are pathogenic to avian species (Taylor *et al.* 2007). Aegyptianellosis is found in all of Africa, southern Europe, the Middle East and the Indian subcontinent (Huchzermeyer 2001). *Aegyptianella pullorum* was named by Carpano (1928), who in the following year (1929) suggested it might be transmitted by the soft tick *Argas persicus* (Oken, 1818) (Bird and Garnham 1969). Three distinct phases can be seen in development of *Ae. pullorum* in the soft tick *Ar. persicus*; development and reproduction in epithelial cells of the intestine, in haemocytes, and in cells of the salivary glands (Gothe and Becht 1969). Trans-ovarial and trans-stadial transmission of *Ae. pullorum* has also been observed in tick vectors (Rikihisa and Kreier 2004). *Argas persicus* can also transmit *Borrelia anserina*, agent of avian borreliosis, which often occurs simultaneously with aegyptianellosis (Hoogstraal 1985). Previously, *B. anserina* was reported from *Ar. persicus* ticks in Lorestan Province, western Iran by the authors (Hosseini-Chegeni *et al.* 2017). Occurrence of avian aegyptianellosis in bird hosts has been reported in different parts of Iran (Dezfoulian *et al.* 2011; Fakhar *et al.* 2013; Mohaghegh *et al.* 2018). To date, no study was designed to detect *Ae. pullorum* in tick vectors in Iran. In the present study, we collected ticks from Lorestan Province, west of Iran. Specimens were identified based on morphological features presented in Hosseini-Chegeni and Tavakoli (2013). DNA of adult ticks was extracted using phenol-chloroform method (Sambrook and Russell 2001). A PCR procedure to amplify a partial fragment of 16S rRNA was performed under a touchdown temperature profile using the primers designed in this study (Faeg: 5′- AGA CGG GTG AGT AAT GC -3′, Raeg: 5′- GTC TGG CAG TAT TAA AAG CA -3′. Amplification program included an initial denaturation of 4 min. at 95°C, 11 cycles of denaturation at 94°C for 50 sec., annealing at 60°C for 60 sec. with 1°C decrease per cycle until 50°C, extension for 60 sec. at 72°C,

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followed by 25 cycles of denaturation at 94°C for 60 sec., annealing at 50°C for 50 sec, extension at 72°C for 60 sec. and a final extension of 72°C for 5 min. The PCR reactions (25 µl) contained 1.5 U of *Taq* DNA polymerase enzyme, 2.5 µl PCR 10x buffer, 2 mM $MgCl_2$, 200 µM dNTPs, 0.5µl forward and reverse primers (10 mM), all ingredients, except primers, are from SinaClon® Co. (Iran); template DNA (50–100 ng/ µl) and 14.8 µl sterile water. The PCR products were visualized on 1% agarose gel electrophoresis under UV light. The 508 bp amplicons were purified using a commercial gel extraction kit (GeneJET, Thermo Fisher Scientific) and were sequenced using the forward primer used in the amplification. Three sequences were edited manually and BLASTed with the sequences deposited in GenBank database. Five PCR positive cases of *Ae. pullorum* were detected (Table 1).

Table 1. Study areas located in Lorestan Province and ticks species were used in this study with related data.

Location	Coordinates	Species	Sex	No. of specimens	PCR positive	Sequencing
Borujerd[1]	33° 57' 9.12" N 48° 57' 41.56" E	*Argas persicus*	Female	4	+	+
Khoramabad[2]	33°18' 33.60" N 48° 42' 54.29" E	*Argas persicus*	Female	4	+	+
Khoramabad[3]	33 °28' 22.03" N 48° 35' 51.86" E	*Argas persicus*	Male	4	+	+
Aleshtar[4]	33° 46' 40.61" N 47° 55' 11.29" E	*Argas persicus*	Female	4	+	-
Pol-e Dokhtar[5]	33° 27' 49.51" N 47° 51' 26.86" E	*Argas persicus*	Male	4	+	-

[1] Darreh Seydi rural district, Kalleh village, [2] Papi district, Sarkaneh village, [3] Zagheh district, Badeh village, [4] Qalayi rural district, Cheshmeh Tala village, [5] Mamulan district, Kur Shurab-e Alishah village

The genetic distance among sequences obtained in this study and seven 16S rRNA sequences of *Aegyptianella*, *Anaplasma*, *Ehrlichia* and *Rickettsia* from the GenBank database were calculated using maximum composite likelihood (MCL) substitution model in MEGA7 software (Kumar *et al.* 2016). So a phylogenetic tree was constructed using Bayesian inferences method (BI) in BEAST software (Ver. 2.4.8) (Fig. 1). The generated sequences were submitted to the GenBank under the accession numbers MH047374-6. *Aegyptianella pullorum* clade that included our sequences and a GenBank 16S rRNA sequence had 2% genetic difference with a clade including *Anaplasma phagocytophilum* (GU064895), *An. bovis* (JN558822) and *Ehrlichia equi* (AF036647). As well as, 6% genetic difference with *E. chaffeensis* (CP007477) and *E. canis* (EF139458). *Rickettsia slovaca* (CP002428) was included as an out-group showing 15% distance difference with *Ae. pullorum* clade. The BLAST results showed the bacteria from the *Ar. persicus* tick is most similar to *Ae. pullorum* only based on 16S rRNA sequence data (Table 2).

Table 2. Similarity of the partial 16SrRNA sequence generated in this study with similar sequences from GenBank database.

Species	Accession number	Identity	Query cover	Total score
Aegyptianella pullorum	AY125087	98	99	802
Anaplasma phagocytophilum	GU064895	98	99	791
Anaplasma bovis	JN558822	98	99	780
Ehrlichia equi	AF036647	97	99	774
Ehrlichia chaffeensis	CP007477	94	99	686
Ehrlichia canis	EF139458	94	99	691
Rickettsia slovaca	CP002428	87	95	484

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Members of the genus *Argas* act as vectors of aegyptianellosis in nature. Some *Anaplasma* spp. were detected from *Argas* ticks (Lafri *et al.* 2017), but to date neither *An. phagocytophilum*, *An. bovis* nor *Ehrlichia* have been detected in *Ar. persicus* ticks. Further studies are required to confirm the detection of bacterial agents in *Ar. persicus* ticks using more specific genetic markers. Though more genetic markers including groEL (HSP) and 23 rRNA were targeted in this study to increase the specificity and reliability of detection of *Ae. pullorum* in soft ticks,unfortunately, PCR amplification failed (groEL using two different primer pairs) or sequencing resulted a non-target *Rickettsia* species (data not shown). Finally, the present paper only reports a preliminary record of *Ae. pullorum* in soft tick vectors, *Ar. persicus* in Iran.

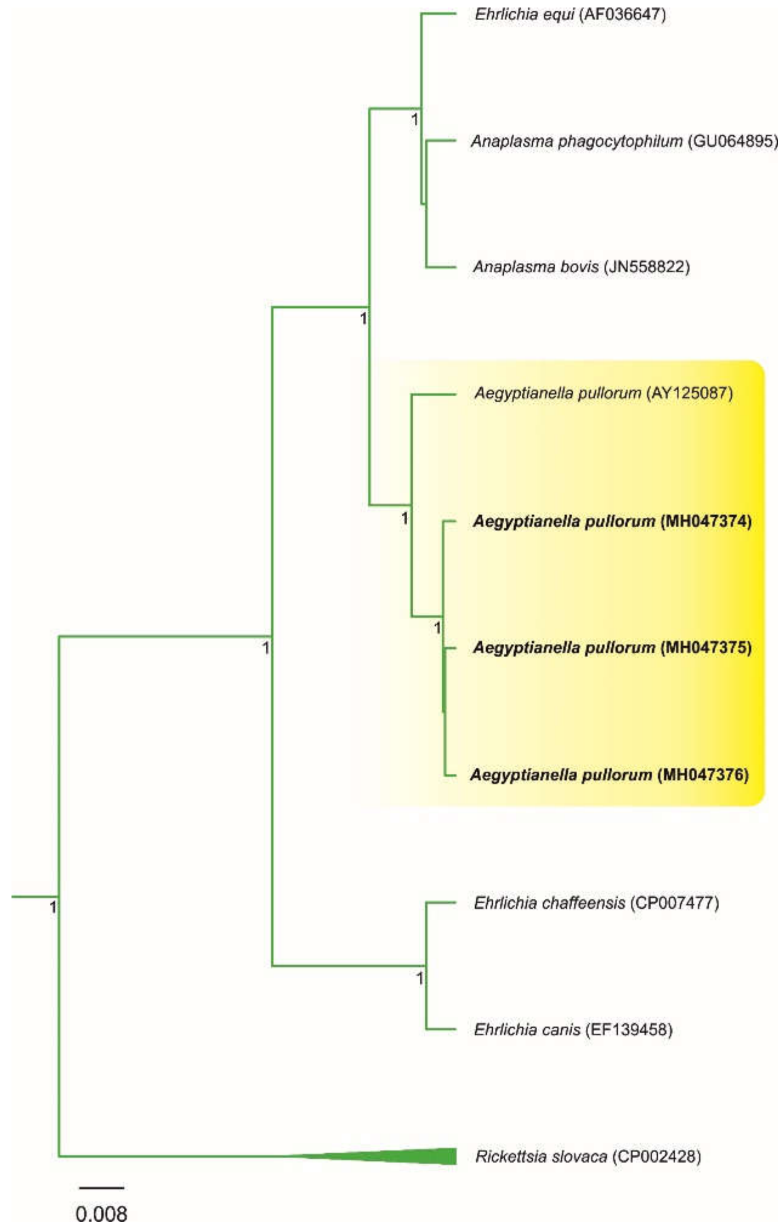

Figure 1. Phylogenetic tree generated based on 16SrRNA sequence data of the *Aegyptianella pullorum* species generated in this study and similar sequences from GenBank database constructed using Bayesian Inference method. Main clade of tree is separated by a rectangular shape. The taxa of the present study are bold and defined with a name and GenBank accession number. Posterior probability values are inserted at nodes. Branch lengths are proportional to the evolutionary changes. Tree is re-rooted by *Rickettsia slovaca* as out-group.

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