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Original article

The geographic distribution of *Argas* (*Persicargas*) *miniatus* and *Argas* (*Persicargas*) *persicus* (Acari: Argasidae) in America, with morphological and molecular diagnoses from Brazil, Chile and Cuba

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ABSTRACT

High similarity of morphological traits has historically overshadowed the identities and distributions of poultry-associated soft ticks *Argas* (*Persicargas*) *miniatus* and *Argas* (*Persicargas*) *persicus* in America. In order to model the occurrence of both parasites in the continent, in the current study we performed morphological and molecular analyses to identify ticks collected in hen houses from Brazil and northern Chile. Combining these results with literature data, and the examination of *Argas* allotments deposited in the tick collections "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (Brazil), the "Coleção Acarológica do Instituto Butantan São Paulo" (Brazil), and the "Colección Zoológica de la Academia de Ciencia de Cuba" (Cuba), we present a critical list with the localities where *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* have been reported in the American continent. Our results confirmed the presence of *A.* (*P.*) *miniatus* in Brazil and Cuba, and *A.* (*P.*) *persicus* in Chile, which in particular, constitutes the first molecularly confirmed report of the later species for South America. Although *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* have been documented in 21 American countries, the identity of some reports must still be considered as uncertain until detailed morphological and/or molecular studies are performed. When contrasted to a Köppen-Geiger climate classification, *A.* (*P.*) *miniatus* predominantly occurs in equatorial and *A.* (*P.*) *persicus* in arid climates. However, until undetermined reports of both species are correctly identified, any conclusion on their geo-climatological occurrence throughout the American continent would be rather speculative.

1. Introduction

Ticks of the genus *Argas* Latreille (Argasidae) are haematophagous parasites in all their postembryonic stages and are currently represented by 61 species distributed in all the Zoogeographic Regions of the world (Guglielmone et al., 2010). Based on a morphological approach of immature and mature stages, taxonomic summaries of this genus have proposed to divide most of its specific diversity in six defined subgenera, namely *Argas*, *Carios*, *Chiropterargas*, *Microargas*, *Persicargas, Secretargas* and an undefined subgenus referring to *Argas bureschi* Dryenski 1957 (Hoogstraal, 1985). Particularly, the *Argas* (*Persicargas*) group is composed by 16 ornithophilous species phenotypically similar to each other (Hoogstraal, 1985; Estrada-Peña et al., 2003), and well adapted to parasitize domestic birds (Hoogstraal, 1956;

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Kohls et al., 1970), to which they can transmit pathogenic eubacteria (Burroughs, 1947; Zaher et al., 1977; Lisbôa et al., 2009) and viruses (Hoogstraal, 1985).

Argas (*Persicargas*) *miniatus* was insufficiently described by Koch (1844) from post-larval stages collected in Guyana (Demerara region), and its identity remained problematical until Kohls et al. (1970) reexamined the type specimens. Based on the anatomy of the peripheral integumental cells of two damaged specimens, these authors concluded that *A.* (*P.*) *miniatus* was a valid taxon, and redescribed the species from previous treatments as a morphological variety (i.e. var. *miniatus* and var. *dissimile*) (Neumann, 1904; Aragão, 1938) and a synonym of *A.* (*P.*) *persicus* (Nuttall et al., 1908). In that same work, by the examination of several collections of immature and mature *Argas* from American localities, the authors presented the distribution of *A.* (*P.*) *miniatus,* including Brazil, Colombia, Guyana, Panama and Trinidad and Tobago (Kohls et al., 1970). The last morphologically confirmed report of this tick species was made from larvae, nymphs and adults collected in Rio Grande do Sul State in Brazil (Evans et al., 2000). Yet, *A.* (*P.*) *miniatus* has been also reported from Venezuela (Vogelsang and Dias, 1953), Cuba (De La Cruz, 1974), Puerto Rico (Capriles and Gaud, 1977), Jamaica and the United States (Keirans, 1984).

The tick *A.* (*P.*) *persicus* was described by Oken (1818) from poultry-associated specimens collected in Iran, yet subsequent reports put in evidence a transcontinental distribution, almost always in association to domestic chicken (Hoogstraal, 1956). To date, *A.* (*P.*) *persicus* has documented reports in all the Zoogeographic Regions of the world with the exception of Antarctica, including the majority of African countries (Hoogstraal, 1956; Cumming, 1999), Australia (Petney et al., 2004), China (Chen et al., 2010), Italy (Pantaleoni et al., 2010) and India (Keirans, 1984). In America, morphological studies have identified this tick from specimens collected in the United Sates, Paraguay (Kohls et al., 1970) and Argentina (Nava et al., 2004). Still uncertain are the reports from Cuba (De La Cruz 1976) and particularly from Chile, where a morphological variety named as *A.* (*P.*) *persicus* var. *porteri* and *A.* (*P.*) *persicus* were documented from the Metropolitan Region and Calama, respectively (Lahille, 1915; Porter, 1928). Both Chilean records were subsequently considered as possible misidentifications with *Argas* (*Argas*) *neghmei* Kohls & Hoogstraal 1961 (Kohls and Hoogstraal, 1961).

The study of the larval phenotype is crucial in order to separate species in the Argasidae family (Hoogstraal, 1985; Klompen, 1992), and might constitute a suitable approach to report new or confirm doubtful records for *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* in America. While the morphology of nymphs and adult stages has shown to be less informative, some features of the dorsal integument constitute useful discrete characters for a specific diagnosis in the *Argas* genus. In order to confirm the identity of *Argas* ticks associated with domestic chicken, in this study we combine morphological and molecular analyses of immature and mature specimens collected in chicken houses from several states of Brazil, the North of Chile and Cuba. Additionally, we present a map with the current distribution and the identity status of the analyzed species in America.

2. Material and methods

2.1. Examined material

Two nymphs, six males and one female collected in Calama (22°27′S; 68°54′W, elevation 2775 m), Antofagasta Region, Chile, date of collection 03 December 2014; 16 larvae, 41 nymphs, 25 females and 22 males collected in Santa Teresinha (07°05′S; 37°26′W; elevation 303 m), Paraiba State, Brazil, date of collection April 2014; and 13 nymphs, 11 females, and five males collected in Brasília (15°37′S; 47°56′W, elevation 1250 m), Distrito Federal, Brazil, date of collection 23 October 2016. All these collections of immature and mature soft ticks were made during daytime inside chicken houses by examining fissures in wooden and concrete-made structures. Subsequently, ticks were transported alive to the laboratory and engorged females were placed in an incubator with 25 °C and 80% of relative humidity in order to obtain ovipositions. In addition to this field-collected material, we examined *Argas* allotments deposited in the three following tick collections: Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva (CNC), Brazil: CNC-878 (two females, four males), CNC-1153 (ten larvae, seven nymphs, seven females, six males), CNC-1154 (five nymphs, ten females, ten males), and CNC-2576 (one nymph, three males, two females); Coleção Acarológica do Instituto Butantan, São Paulo (IBSP), Brazil: IBSP-731 (five nymphs, four females, one male), IBSP-803 (two females, one male), IBSP-863 (one female, two males), IBSP-937 (two nymphs, 12 females, ten males), IBSP-1267 (two nymphs, four females), IBSP-1269 (26 females, ten males), IBSP-1279 (seven females, two males), IBSP-1746 (two females, seven males), IBSP-4214 (seven nymphs, six females, five males), IBSP-4317 (28 nymphs, 18 females, 12 males), IBSP-4513 (one nymph, ten females, one male), IBSP-5954 (two males), IBSP-9251 (two females, one male), and IBSP-9934 (one nymph, two males); and the Colección Zoológica de la Academia de Ciencia de Cuba (CZACC), Cuba: N°10704 (one nymph, nine females, six males), and N°10734 (four nymphs, seven females, eight males).

2.2. Morphological analyses

Two cohorts of ten laboratory-reared larvae, each one obtained from a unique female per locality, and ten larvae, 12 females and one male from the CNC were clarified in a 20% KOH solution, and mounted in slides using Hoyer's medium. In order to visualize the setal pattern with detail and to compare with the original description of other Argasinae, the capitulum of adult specimens was mounted apart from the idiosoma, following the dissection methodology proposed by Cooley and Kohls (1944). Morphological characters of slide-mounted larvae (Fig. 1) and adults were observed under light microscopy and measured above micrographs using the software Image-Plus Pro v5.1. Post-larval specimens from the CNC, CZACC and IBSP were examined using a SteREO Discovery V12 stereomicroscope and measured with the software ZEN 2 pro. All measurements are given in millimeters with the standard deviation followed by the range in parenthesis. We adopted morphological definitions of Sonenshine et al. (1962) for larvae of *Argas* genus, which consider that only the subgenus *Chiropterargas* has dorsolateral setae arranged in an anterior and posterior groups. As a clear anatomical definition of both groups of setae is not specified either for *A.* (*P.*) *miniatus* or *A.* (*P.*) *persicus*, we did not consider these characters in our study and rather obtained averages of dorsolateral setae.

In order to discriminate the relationship between morphologically similar species, obtained measures of unengorged larvae were submitted to a principal component analysis (PCA) based on Pearson correlation matrix for 40 morphological variables (Table 1). Measurements of *Argas* (*Persicargas*) *keiransi* Estrada-Peña, Venzal & González-Acuña 2003 were also included in the comparisons.

To observe in detail morphological characters with specific significance, one female of the CZACC was prepared for electron microscopy photographs following Corwin et al. (1979). Subgenus level determination using larval stages followed Kaiser et al. (1964). Species level identification were performed by comparing the obtained morphological and morphometrical data with original descriptions (Kohls and Hoogstraal, 1961; Estrada-Peña et al., 2003) and redescriptions of Argasinae from America (Kohls et al., 1970).

2.3. Molecular tools

To confirm morphological diagnoses, DNA extraction using the Guanidine Isothiocyanate technique (Sangioni et al., 2005) was individually performed in three females, three nymphs and two larvae from Calama (Chile), one male and two larvae from Santa Teresinha

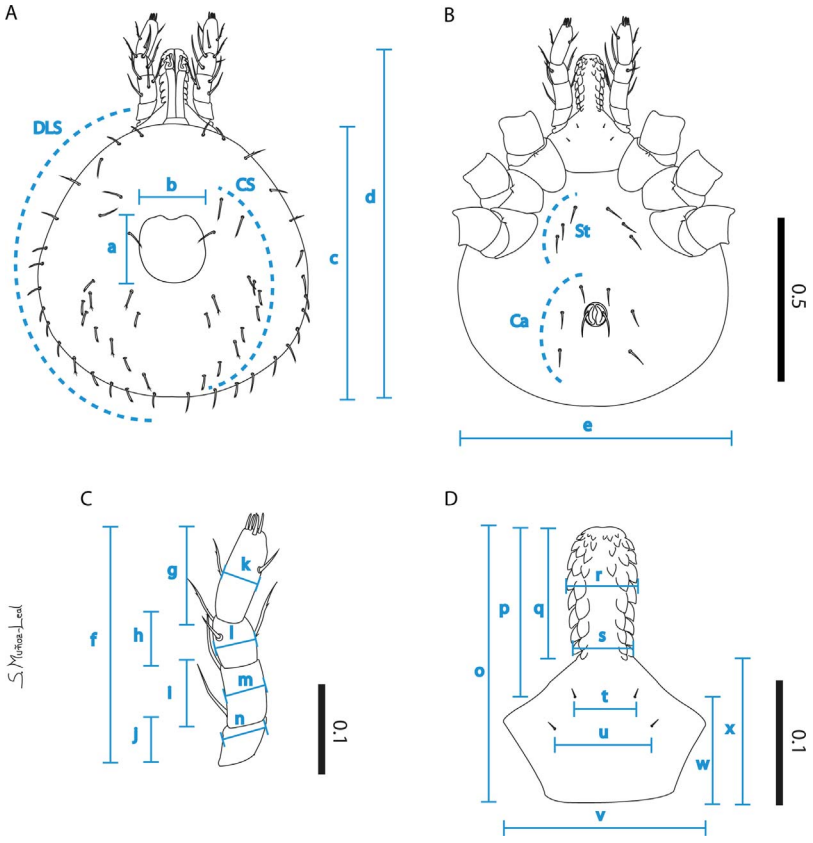

Fig. 1. Measurements and setal groups of unengorged larval stages examined in this study (light blue). (A) Dorsal view. Dorsal plate: (a) length, (b) width. Body: (c) length not including capitulum, (d) length including capitulum. (B) Ventral view: (e) body width. (C) Palpus: (f) total length; length of articles: (g) article I, (h) article II, (i) article III, and (j) article IV. Width of articles: (k) article I, (l) article II, (m) article III, and (n) article IV. (D) Ventral capitulum: (o) total length; hypostome: (p) length measured to ph1, (q) length measured to insertion, (r) middle width, and (s) base width; basis capitulum: (t) distance between ph1 setae, (u) distance between ph2 setae, (v) width, (w) length to insertion of hypostome, and (x) length to insertion of hypostome. Groups of setae are denoted with a doted line. Scale bars are given in mm. Abbreviations: DLS, dorsolateral setae; CS, central setae; St, sternal setae; Ca, circumanal setae. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Brazil), one female and one nymph from Brasilia (Brazil), two females and six males from the CNC (Brazil), and one female and one nymph from the CZACC (Cuba). A conventional PCR to amplify a ≈460-bp fragment of the tick mitochondrial 16S rRNA gene was performed (Mangold et al., 1998). Expected size amplicons were purified with ExoSAP-IT® and further sequenced using the BigDye® Terminator v3.1 Cycle Sequencing (Applied Biosystems, Austin, USA) in an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, CA) with the same primers used for PCR. Generated sequences were assembled and primer-trimmed with Geneious R9 software (Kearse et al., 2012), and then submitted to BLAST analyses (www.ncbi.nlm.nih.gov/blast) in order to infer closest similarities with other argasid species available in GenBank.

2.4. Phylogenetic analysis

An alignment of a partial fragment of the 16S rDNA mitochondrial gene was constructed with 19 *Argas* sequences retrieved from GenBank (AB819157, AF001401, AF001402, AF001403, AF001404, AY436768, AY436772, DQ295778, DQ295781, EU283344, GU355921, GU451248, KC769587, KC769590, L34305, L34321, NC019642, NC029175, and U95863), and eight obtained in the present study. The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999), with manual edition whenever necessary, and aligned with the program Clustal W (Thompson et al., 1994). Phylogenetic relationships were assessed with the maximum-likelihood (ML) method and the best-fitting substitution model was determined with the Bayesian Information Criterion using MEGA 5 (Tamura et al., 2011). GTR model was chosen to create ML trees. Branch support was tested by bootstrap analysis using 1000 replicates. Sequences of *Ornithodoros brasiliensis* Aragão, 1923 (GU198368) and *Ornithodoros rostratus* Aragão, 1911 (DQ295780) were used as out-group.

2.5. Literature revision and mapping

We listed in a table all the documented reports of *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* for America published until 2016, including unpublished data from the CNC, IBSP and original records of this study. Reports for the United States listed by Bishopp (1941), Cooley and Kohls (1944), and Bishopp and Trembley (1945) were not included, since Kohls et al. (1970) synonymized the majority of its referred collections either with *Argas* (*Persicargas*) *radiatus* Raillet, 1893 or *Argas* (*Persicargas*) *sanchezi,* Dugès 1887. As immature and mature stages of *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* are morphologically closely related, some reports have been considered as possible misidentifications (Kohls et al., 1970; Guglielmone and Nava, 2005). Therefore, we regarded as valid all the localities where the identification of ticks was based either on a molecular or on an explicit morphological analysis, noting the specific characters that separate both *Argas* (*Persicargas*) species. Reports lacking this methodology were considered as pending confirmation. Geographical coordinates of non-georeferenced localities were obtained using GEOLocate v.3.21 (Rios and Bart, 2010). When the exact locality of tick collection was not given in the original article, geographic coordinates for the centroid of the country or administrative division indicated as geographic reference in each published record, were calculated based in shapes files retrieved from DIVA-GIS (2011) using the software QGIS 2.16.2 (Quantum GIS Devolpment Team, 2016). Subsequently, all the compiled locations were plotted over a Köppen-Geiger climate classification map (Kottek et al., 2006) based on the R-code given by Rubel et al. (2017).

3. Results

3.1. Morphological study

By the morphological analysis of 30 slide-mounted larvae, six slide-mounted females, late-stage nymphs and adult specimens, two species

Table 1
Average and range (mm) of *Argas* (*Persicargas*) *miniatus*, *Argas* (*Persicargas*) *persicus* and data obtained from literature. Characters used to perform the Principal Components Analysis are denoted with an asterisk.

	A. (P.) miniatus			*A. (P.) persicus*	
	Paraiba State, Brazil (This study)	Espírito Santo State, Brazil (This study)	Kohls et al. (1970)	Calama, Chile (This study)	Kohls et al. (1970)
Feeding status	Unengorged	Unengorged	Unengorged	Unengorged	Unengorged
Body length including capitulum*	0.892 ± 0.015 (0.871–0.913)	0.881 ± 0.010 (0.866–0.896)	0.760–0.870	0.975 ± 0.024 (0.944–1.022)	0.950–0.980
Body length not including capitulum*	0.603 ± 0.012 (0.586–0.597)	0.605 ± 0.011 (0.587–0.622)	–	0.764 ± 0.013 (0.741–0.783)	–
Body width*	0.614 ± 0.010 (0.597–0.629)	0.624 ± 0.022 (0.582–0.645)	0.530–0.730	0.788 ± 0.019 (0.762–0.819)	0.680–0.740
Dorsal plate: form	Oval	Oval	Oval	Circular	Circular
Dorsal plate: length*	0.200 ± 0.007 (0.187–0.211)	0.203 ± 0.007 (0.190–0.211)	0.188–0.220	0.163 ± 0.014 (0.143–0.185)	0.200–0.240
Dorsal plate: width*	0.159 ± 0.004 (0.154–0.166)	0.155 ± 0.006 (0.147–0.169)	0.148–0.180	0.171 ± 0.008 (0.158–0.182)	0.180–0.200
Dorsal setae: total pairs*	28–29	28–29	25–31	26–28	26–29
Dorsal setae: dorsolateral pairs*	14	14	14	15	14–16
Dorsal setae: central pairs*	14–15	14–15	11–17	11–13	11 −13
DAL setae media	–	–	0.049	–	0.038
DPL setae media	–	–	0.070	–	0.039
DL setae media	0.052 ± 0.002 (0.051–0.055)	0.051 ± 0.002 (0.048–0.054)	–	0.041 ± 0.001 (0.040–0.043)	–
C setae media*	0.038 ± 0.001 (0.036–0.040)	0.037 ± 0.002 (0.033–0.039)	–	0.042 ± 0.002 (0.040–0.044)	–
Ventral setae (pairs): total	6 + anal pair	6 + anal pair	6 + anal pair	6 + anal pair	6 + anal pair
Sternal setae: St1*	0.045 ± 0.003 (0.040–0.049)	0.046 ± 0.002 (0.041–0.049)	–	0.043 ± 0.003 (0.039–0.047)	–
Sternal setae: St2*	0.047 ± 0.002 (0.044–0.050)	0.047 ± 0.004 (0.042–0.052)	–	0.043 ± 0.003 (0.038–0.047)	–
Sternal setae: St3*	0.048 ± 0.005 (0.034–0.052)	0.049 ± 0.004 (0.043–0.057)	–	0.043 ± 0.002 (0.039–0.047)	–
Circumanal setae: Ca1*	0.046 ± 0.005 (0.038–0.053)	0.046 ± 0.004 (0.040–0.052)	0.046	0.053 ± 0.002 (0.051–0.057)	0.049
Circumanal setae: Ca2*	0.049 ± 0.004 (0.041–0.056)	0.047 ± 0.004 (0.041–0.053)	0.048	0.049 ± 0.005 (0.040–0.056)	0.045
Circumanal setae: Ca3*	0.049 ± 0.005 (0.037–0.054)	0.046 ± 0.004 (0.038–0.053)	0.045	0.051 ± 0.004 (0.042–0.055)	0.048
Posteromedian setae: Pms	absent	absent	absent	absent	absent
Postcoxal setae: Pc	absent	absent	absent	absent	absent
Anal plate setae: As	0.052 ± 0.005 (0.037–0.054)	0.053 ± 0.005 (0.046–0.063)	–	0.067 ± 0.005 (0.060–0.077)	–
Length of basis capituli[a] *	0.122 ± 0.006 (0.112–0.132)	0.122 ± 0.008 (0.110–0.133)	–	0.113 ± 0.007 (0.104–0.126)	–
Length of basis capituli[b] *	0.182 ± 0.011 (0.169–0.200)	0.180 ± 0.008 (0.163–0.191)	–	0.145 ± 0.007 (0.130–0.154)	–
Length of capituli*	0.340 ± 0.007 (0.326–0.350)	0.337 ± 0.008 (0.323–0.348)	0.300–0.340	0.308 ± 0.007 (0.296–0.319)	0.310
Width of basis capituli*	0.269 ± 0.008 (0.256–0.279)	0.272 ± 0.012 (0.241–0.281)	0.200–0.230	0.230 ± 0.008 (0.219–0.241)	0.200–0.240
Posthypostomal setae Ph1	0.007 ± 0.001 (0.006–0.008)	0.007 ± 0.002 (0.004–0.009)	0.006	0.005	0.004
Posthypostomal setae Ph2	0.009 ± 0.002 (0.006–0.010)	0.009 ± 0.002 (0.007–0.012)	0.006	0.005	0.004
Distance Ph1–Ph1	0.071 ± 0.003 (0.065–0.075)	0.074 ± 0.002 (0.069–0.078)	0.084	0.076 ± 0.004 (0.067–0.079)	0.077
Distance Ph2–Ph2	0.116 ± 0.005 (0.109–0.123)	0.121 ± 0.005 (0.113–0.128)	0.131	0.122 ± 0.005 (0.116–0.130)	0.138
Palpal length*	0.262 ± 0.006 (0.251–0.270)	0.263 ± 0.006 (0.250–0.273)	0.230–0.260	0.281 ± 0.006 (0.268–0.287)	0.270–0.280
Length article I*	0.054 ± 0.007 (0.040–0.063)	0.053 ± 0.004 (0.048–0.059)	0.052–0.068	0.055 ± 0.004 (0.049–0.064)	0.056–0.080
Length article II*	0.071 ± 0.006 (0.059–0.079)	0.070 ± 0.003 (0.063–0.073)	0.060–0.076	0.073 ± 0.006 (0.068–0.084)	0.068–0.076
Length article III*	0.054 ± 0.004 (0.049–0.061)	0.051 ± 0.002 (0.047–0.054)	0.056–0.064	0.065 ± 0.003 (0.061–0.070)	0.056–0.068
Length article IV*	0.103 ± 0.003 (0.097–0.107)	0.104 ± 0.003 (0.099–0.109)	0.096–0.120	0.117 ± 0.005 (0.109–0.128)	0.116–0.128
Width artcile I*	0.047 ± 0.005 (0.039–0.056)	0.046 ± 0.004 (0.041–0.053)	–	0.064 ± 0.005 (0.057–0.075)	–
Width article II*	0.050 ± 0.002 (0.047–0.054)	0.054 ± 0.004 (0.050–0.062)	–	0.050 ± 0.003 (0.048–0.057)	–
Width article III*	0.050 ± 0.003 (0.044–0.053)	0.051 ± 0.002 (0.047–0.054)	–	0.049 ± 0.002 (0.046–0.052)	–
Width article IV*	0.044 ± 0.002 (0.042–0.048)	0.044 ± 0.002 (0.041–0.047)	–	0.043 ± 0.001 (0.040–0.045)	–
N° setae article I	0	0	0	0	0
N° setae article II	5	5	5	5	5
N° setae article III	4	4	4	4	4
N° setae article IV	12	12	12	12	12
Hypostome[c] *	0.221 ± 0.008 (0.205–0.231)	0.217 ± 0.005 (0.208–0.228)	–	0.197 ± 0.005 (0.192–0.206)	–
Hypostome[d]	0.161 ± 0.006 (0.148–0.169)	0.156 ± 0.006 (0.146–0.168)	–	0.161 ± 0.003 (0.157–0.165)	–
Hypostome[e] *	0.161 ± 0.006 (0.148–0.169)	0.156 ± 0.006 (0.146–0.168)	0.140–0.160	0.161 ± 0.003 (0.157–0.165)	0.168
Hypostome: base width*	0.093 ± 0.008 (0.082–0.108)	0.097 ± 0.004 (0.088–0.103)	–	0.077 ± 0.005 (0.069–0.083)	0.080–0.084
Hypostome: middle width*	0.095 ± 0.003 (0.090–0.100)	0.089 ± 0.005 (0.082–0.096)	–	0.082 ± 0.003 (0.077–0.089)	–
Apex	blunt		blunt	blunt	blunt
Apical dental formula (corona)*	3/3–4/4	3/3–4/4	3/3	3/3	3/3
Median dental formula*	2/2	2/2	2/2	2/2	2/2
Basal dental formula*	2–2	2–2	2/2	2/2	2/2
Denticles in hypostomal row 1*	7–9	7–8	8–11	9–10	9–10
Denticles in Hypostomal row 2*	7	6–7	7–10	7–8	6–9
Denticles in Hypostomal row 3*	1–3	1–2	2–5	1–3	1–5
Tarsus I: length*	0.291 ± 0.010 (0.271–0.307)	0.311 ± 0.007 (0.297–0.319)	0.248–0.290	0.312 ± 0.008 (0.304–0.323)	0.300–0.310
Tarsus I: width*	0.086 ± 0.007 (0.077–0.102)	0.089 ± 0.005 (0.082–0.096)	–	0.080 ± 0.002 (0.007–0.083)	–

[a] Length measured to Ph1 insertion.
[b] Length measured to the insertion of hypostome.
[c] Length measured to Ph1 insertion.
[d] Length measured to point of inferior toothed portion.

[e] length measured to the insertion.

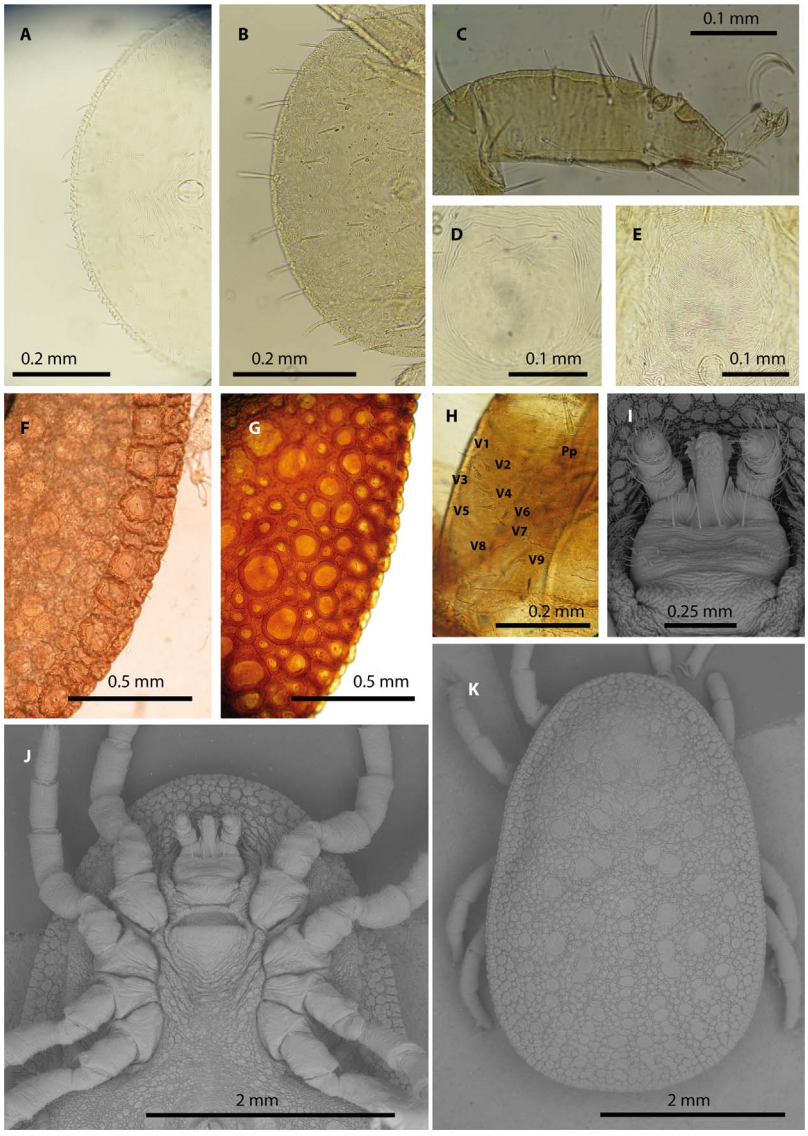

Fig. 2. Light and scanning electron microscopy micrographs of *Argas* (*Persicargas*) *miniatus* and *Argas* (*Persicargas*) *persicus*. *Argas* (*P.*) *persicus*: larva: (A) posterior portion of ventral idiosoma, and (D) dorsal plate; adult: (F) lateral integumental cells. *Argas* (*P.*) *miniatus*: larva: (B) posterior portion of ventral idiosoma, (C) tarsus I, and (E) dorsal plate; adult: (G) lateral integumental cells, (H) ventral basis capitulum, (I) ventral capitulum, (J) ventral view and (K) dorsal view. Abbreviations: V, ventral setae; Pp, postpalpal seta.

of *Argas* (*Persicargas*) were recognized. Chilean specimens were diagnosed as *A.* (*P.*) *persicus* (Oken, 1818) by the following combination of characters: Larva – dorsal plate subcircular, dorsal setae short, 14–16 dorsolateral setal pairs, nearly equal in length (≈0.039 mm); tarsus I setal formula: 1 apical pair, 3 paracapsular setae, 2 posteromedian, 1 basal, 1 apicoventral, 1 midventral, 1 posterolateral, and 2 basoventral pairs; Haller's organ without coralline reticulations and lacking a trumpet like sensillum (Kohls et al., 1970) (Fig. 2A, D). Female – a row with less than 100 large rectangular or subrectangular peripheral cells, adjacent one to another or separated either by a narrow deep groove or by a small irregular ridge; each cell provided with a large stout seta (Kohls et al., 1970) (Fig. 2F); measurements of five analyzed females were as follow (this study/Kohls et al., 1970): 6.231–8.447 (7.289 ± 1.042)/5.10–9.75 mm length, and 3.741–5.019 (4.434 ± 0.552)/3.40–6.15 mm width.

Argas (*P.*) *miniatus* was identified from field-collected material in Brazil, and also in CNC, CZACC and IBSP allotments by the following combination of characters: Larva – dorsal plate oval, dorsum provided with 14 dorsolateral setal pairs, 11–17 central setal pairs; tarsus I setal formula: 1 apical pair, 3 paracapsular setae, 1 pair posteromedian, 1 basal, 1 apicoventral, 1 midventral, 1 posterolateral, and 2 basoventral pairs; Haller's organ without coralline reticulations and lacking a trumpet like sensillum (Kohls et al., 1970) (Fig. 2B, C, E). Female – a row with more than 150 medium sized subrectangular, subtriangular and subcircular peripheral cells, adjacent to one another, each cell provided with one or two setal pits; basis capituli provided with 9–12 pairs of ventral setae (Kohls et al., 1970) (Fig. 2G–J); measurements of five females were as follow (CNC/Kohls et al., 1970): 5.137–6.448 (5.703 ± 0.586)/4.300–7.700 mm length and 3.256–4.064 (3.610 ± 0.344)/2.770–4.920 mm width.

A comparison of larval morphometry obtained in this study with data from literature is presented in Table 1.

Vouchers in ethanol and slide-mounted specimens were deposited in the "Coleção Nacional de Carrapatos Danilo Saraiva" (CNC) at the Veterinary Medicine and Zoothecnics Faculty in University of São Paulo, São Paulo, Brazil under the following accession numbers: CNC-878, −1153, −1154, −3376, −3382, −3409 for *A.* (*P.*) *miniatus,* and CNC-3299, −3302 for *A.* (*P.*) *persicus*.

3.2. PCA

Morphological comparison through the PCA showed a clear

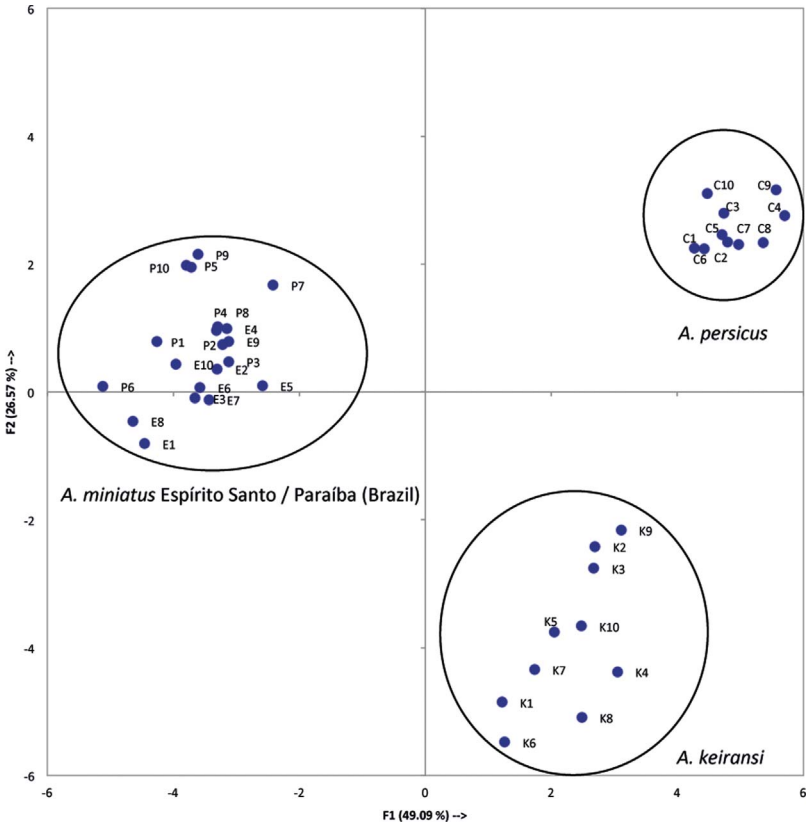

Fig. 3. Principal components analysis of *A*. (*P*.) *miniatus* from Espírito Santo state (E 1–10) and Paraíba state (P 1–10); *A*. (*P*.) *persicus* from Calama (C 1–10), and *A*. (*P*.) *keiransi* (K 1–10), using 40 morphological characters detailed in Table 1. Each point constitutes the position of each measured specimen on the reduced space.

difference between both *Argas* species diagnosed in the current study and also with *A*. (*P*.) *keiransi* (Fig. 3). The first principal component, which in this case explains 49.09% of the total variance, was almost fully loaded with the following characters: body length (not including capitulum), body width, hypostome length (measured to ph1), hypostome width and palpal length (articles III and IV). On the other hand, the second component explained 26.57% of the total variance, and heavier characters were represented by length of dorsal plate, dorsal setae (total pairs), central setae and tarsus I width.

3.3. Molecular tools

Eight ticks from Chile submitted to DNA extraction yielded identical sequences among each other and the consensus of 407-bp was deposited in GenBank under the accession number KX258880. Thirteen ticks from Brazil yielded two haplotypes of 400-bp differing in one nucleotide. One haplotype was obtained from ticks collected in Santa Teresinha (Paraiba), São Roque de Minas (Minas Gerais) and Santa Cruz do Ibitinema (Rio de Janeiro), and a second haplotype was retrieved from ticks collected in Paraiba do Sul (Rio de Janeiro) and São Mateus (Espírito Santo). Ticks from Brasília (Federal District) yielded both haplotypes. Our attempts to amplify tick DNA in both Cuban specimens were unsuccessful. Sequences from Brazil were deposited in GenBank under the accession numbers KX855206 – KX855210, KY705380 and KY705381. By BLAST analyses we confirmed our morphological diagnoses, since partial mitochondrial 16S rDNA sequences obtained from Chilean ticks were 100% (407-bp) identical to *A*. (*P*.) *persicus* from Italy (GU451248), and the Brazilian ticks yielded sequences 99% (399/400-bp) – 100% identical to *A*. (*P*.) *miniatus* (KC769590).

3.4. Phylogenetic analysis

The phylogenetic tree clearly shows representatives of *Argas* (*Persicargas*) grouping in a separated clade supported by high bootstrap. Within this branch, *A*. (*P*.) *miniatus* formed a clade with *Argas* (*Persicargas*) *walkerae* Kaiser & Hoogstraal, 1969, and *A*. (*P*.) *keiransi* appeared as a sister taxon of *A*. (*P*.) *persicus* (Fig. 4).

3.5. Literature revision and mapping

Both *Argas* species have been reported in 145 American localities. *Argas* (*P*.) *persicus* has documented collections in 45 localities from North America, and in 25 from South America. In turn, documented distribution of *A*. (*P*.) *miniatus* includes 65 localities in South America, and 10 reports from North America (Table 2). Overall, 69% (101/145) of the reports lack a reliable morphological or molecular analysis for the species diagnosis. Confirmed reports for both soft tick species fall within arid, equatorial, and warm temperate climate regimes, however records of *A*. (*P*.) *persicus* also reach desert ecosystems (Fig. 5).

4. Discussion

Species diagnosis of immature and mature *Argas* (*Persicargas*) ticks constitutes a challenging task (Khalil et al., 1980). Setal distribution and dimensions in the dorsal and ventral faces of larvae are often similar and, in these cases, differences of micrometers are assumed to separate the species (Sonenshine, 1962; Hoogstraal et al., 1968; Kohls et al., 1970). In adults, important differences are described upon the fine anatomy of the dorsal integument, including the form and disposition of peripheral cells (Kohls et al., 1970). Therefore, taking into account morphological similarities within species of *Argas* (*Persicargas*) group, a molecular characterization is suitable to confirm previous diagnoses.

4.1. Morphological study

Larval and adult morphology of *Argas* sp. collected in Brazil was concordant with the redescription of *A*. (*P*.) *miniatus*. Although a

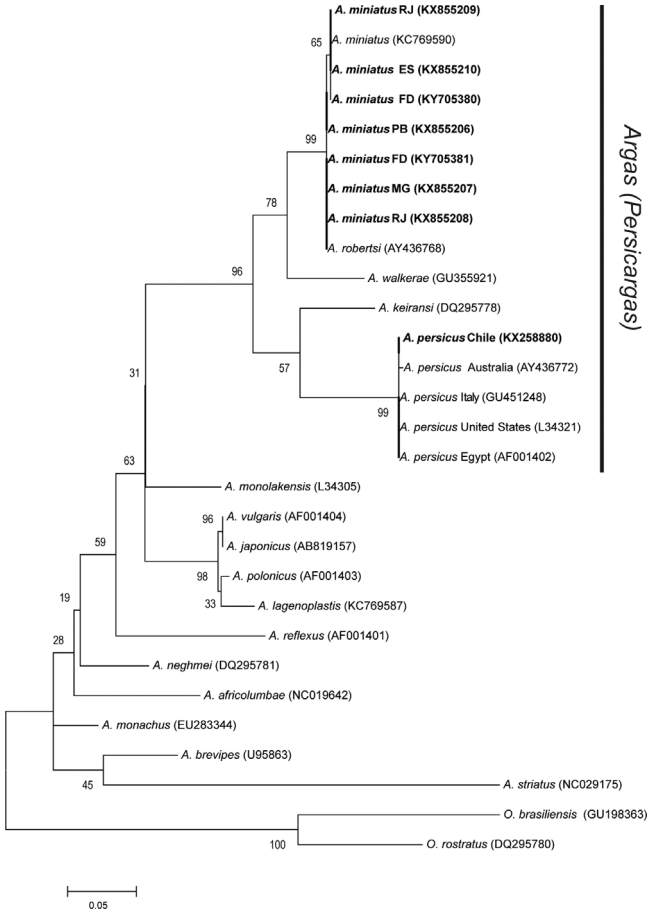

Fig. 4. Phylogenetic tree of *Argas* spp. The sequences obtained in the current study are highlighted in bold. Abbreviations: *A.*, *Argas*; *O.*, *Ornithodoros*; ES, Espírito Santo state; FD, Federal District; MG, Minas Gerais state; PB, Paraiba state, RJ: Rio de Janeiro state.

specific diagnosis by molecular tools was not possible, probably by an advanced state of DNA degradation, the morphological identification of adults *A.* (*P.*) *miniatus* from Cuba was supported by the combination of 9–12 ventral pairs of setae in the capitulum, the anatomy of the dorsal tegument, and the presence of more than 150 peripheral cells (Fig. 2I, K). These morphological differences also separate examined Cuban *Argas* from *A.* (*P.*) *radiatus* and *A.* (*P.*) *sanchezi,* since both later species present more than 16 pairs of ventral setae in the capitulum (Kohls et al., 1970).

All the measured characters of *A.* (*P.*) *persicus* larvae obtained from Chilean specimens are concordant with the dimensions given in the species redescription, with the exception of dorsal plate, which was smaller in our specimens (0.163 mm length–0.171 mm width). Differences in the dimensions of this character were also noticed by Kohls et al. (1970), since larvae from the United States (Pennsylvania) had a smaller dorsal plate when comparing with larval specimens from other origin. Considering this variability, it seems that the dorsal plate could be a polymorphic character uninformative when performing specific morphological diagnoses, at least for this species.

When comparing measurements of *Argas* (*Persicargas*) obtained by our methodology (Fig. 1) by means of a PCA analysis, heavier characters that separate species are represented by the size of the body, hypostome width and length of articles III and IV. This suggests that these traits possess enough interspecific variability, and could correspond to a valuable set for performing morphological comparisons with the rest of *Argas* (*Persicargas*) species in order to discriminate between taxa.

In the current study, the nomenclature of characters proposed by Sonenshine et al. (1962) was considered for analyzing slide-mounted larvae. These authors stated that only for *Chiropterargas* subgenus dorsolateral setae were arranged in anterior (DAL setae) and posterior groups (DPL setae), and that for the rest of subgenera, no evident separation of these traits was observed. Conversely, while posterior descriptions of *Argas* species did include averages of DAL and DPL setae, a clear anatomical definition noting the number of setae for each group was not specified. This fact is evidenced in the descriptions of *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* larval stages (Kohls et al., 1970), and prompted the exclusion of these characters in our morphological analyses.

4.2. Molecular analyses

While the mitochondrial 16S rDNA sequences of Brazilian *A.* (*P.*) *miniatus* characterized in this study confirmed the identity of the species, it was also evidenced that *Argas* (*Persicargas*) *robertsi* Hoogstraal, Kaiser & Kohls, 1968 from Australia has a mitochondrial 16S rDNA sequence 99–100% similar to *A.* (*P.*) *miniatus* from Brazil. This intriguing coincidence between two *Argas* (*Persicargas*) species with vastly distanced geographical distributions was already pointed by Burger et al. (2014). These authors noted that either *A.* (*P.*) *robertsi* was present in America, being *A.* (*P.*) *miniatus* a local race of the Australian tick, or the GenBank number of *A.* (*P.*) *robertsi* (AY436768) was misidentified. Although we do not discard the second possibility, to assume that both *Argas* correspond rather to one geographically widespread species needs further foundation. Still, it is noteworthy to mention that morphological traits of larvae and adults stages of both soft ticks are extremely similar, a fact that complicates even more the scenario. While genetic and morphological similarity in both species constitutes a problematic question yet to be solved, a detailed morphological study, the characterization of less conserved genes and an assessment of reproductive isolation mechanisms would constitute useful approaches in order to decide their validity as taxa.

Obtained mitochondrial 16S rDNA sequence of the Chilean *A.* (*P.*) *persicus* was identical to conspecific sequences characterized from Italian specimens (Pantaleoni et al., 2010), and 99% identical with *A.* (*P.*) *persicus* from Australia and the United States. As this tick species is a common parasite of poultry (Kohls et al., 1970; Hoogstraal, 1985; Petney et al., 2004), it is reasonable to say that its entrance to Chile could have been via infested chickens. *Argas* (*P.*) *persicus* is an originally Palaearctic species spread to many parts of the world (Hoogstraal, 1985, 1956), and constitutes a recognized poultry parasite of medical importance. It has been documented as a vector of *Borrelia anserina* and *Aegyptianella pullorum*, implicated in the circulation of West Nile virus and also in persistent infections of *Salmonella gallinarum* and *Salmonella pullorum* (Stefanov et al., 1975; Hoogstraal, 1985). Moreover, *Rickettsia* spp. of the Spotted Fever and Transitional groups have been reported in *A.* (*P.*) *persicus* from Europe (Rehácek et al., 1977) and Africa (Pader et al., 2012), respectively. However, the vector competence of this tick in relation with these microorganisms must still be assessed.

4.3. Geographical distribution in America

Argas (*Persicargas*) ticks associated with domestic chicken have been widely reported in America. Documented records of *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* were published in taxonomical works (Nuttall et al., 1908; Cooley and Kohls, 1944; Kohls et al., 1970), list of species or reports for a specific region (Hooker, 1909; Rohr, 1909; Lahille, 1915; Dunn, 1923; Vogelsang, 1928; Vigueras, 1934; Aragão, 1935, 1936, 1938; Hearle, 1938; Osorno-Mesa, 1940; Vogelsang and Cordero, 1940; Bishopp and Trembley, 1945; Boero, 1945; Hoffmann, 1962; Fairchild et al., 1966; De La Cruz, 1974, 1976; Capriles and Gaud, 1977; Keirans, 1984; Need et al., 1991; Ivancovich and Luciani, 1992; Evans et al., 2000; Nava et al., 2004; Guglielmone and Nava, 2005; Acosta et al., 2016; Castillo-Martínez et al., 2016), biological studies (Lorosa et al., 2007; Santos et al., 2010), and research related to *Borrelia anserina*

Table 2
List of localities where *Argas* (*Persicargas*) *miniatus*, *Argas* (*Persicargas*) *persicus* have been reported in America. Abbreviations: RPC, report pending confirmation; CR, confirmed report; CNC, Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva; CZACC, Colección Zoológica de la Academia de Ciencia de Cuba; IBSP, Instituto Butantan São Paulo.

Species	Country	Administrative division	Locality	Latitud	Longitud	Host	Reference	Observations
Argas (P.) persicus	Antigua and Barbuda	Antigua	Unknown	19°17′56”N	68°47′33”W	Unknown	Cooley and Kohls (1944)	RPC
	Argentina	Buenos Aires	Hurlingham	34°35′28”S	58°37′33”W	Unknown	Aragão (1938)	RPC
			Luján	34°34′01”S	59°06′39”W	*Gallus gallus domesticus*	Boero (1945)	RPC
			Ituzaingó	34°39′38”S	58°40′27”W	*Gallus gallus domesticus*	Boero (1945)	RPC
		Córdoba	Cruz del Eje	30°43′21”S	64°48′28”W	*Gallus gallus domesticus*	Boero (1945)	RPC
		Entre Rios	Colón	31°13′31”S	58°08′35”W	*Gallus gallus domesticus*	Boero (1945)	RPC
			Rosario del Tala	32°18′13”S	59°08′32”W	*Gallus gallus domesticus*	Boero (1945)	RPC
			Villaguay	31°51′56”S	59°01′42”W	*Gallus gallus domesticus*	Boero (1945)	RPC
		Formosa	El Colorado	26°18′26”S	59°22′26”W	*Gallus gallus domesticus*	Ivancovich and Luciani (1992)	RPC
		San Juan	Albardón	31°31′59”S	68°32′04”W	*Gallus gallus domesticus*	Aragão (1935)	RPC
			San Juan	31°32′09”S	68°32′05”W	*Gallus gallus domesticus*	Boero (1945)	RPC
		San Luis	Unknown	39°57′50“S	73°30′16“W	*Zaedyus pichi* burrow	Aragão (1938)	RPC
		Santa Fe	La Gallareta	29°35′26”S	60°22′39”W	Unknown	Aragão (1938)	RPC
			Tostado	29°13′49”S	61°48′13”W	*Gallus gallus domesticus*	Boero (1945)	RPC
			Rosario	32°57′28”S	60°39′07”W	*Gallus gallus domesticus*	Boero (1945)	RPC
	Canada	Vancouver	British Columbia	49°14′20”N	123°00′26”W	*Zonotrichia atricapilla*	Hearle (1938)	RPC
	Chile	Atacama	Calama	22°27′41”S	68°54′25”W	*Gallus gallus domesticus*	This study	CNC 3302, 3299
		Metropolitan Region	San Bernardo	33°35′13”S	70°42′02”W	Grass	Lahille (1915)	RPC
	Colombia	Atlántico	Barranquilla	10°57′50”N	74°47′47”W	*Gallus gallus domesticus*	Osorno-Mesa (1940)	RPC
	Cuba	Havana	Unknown	26°29′13“N	91°41′15“W	*Gallus gallus domesticus*	Vigueras (1934)	RPC
		Matanzas	Unknown	26°22′27“N	90°49′08“W	*Gallus gallus domesticus*	Vigueras (1934)	RPC
		Santa Clara	Unknown	25°33′00“N	88°59′14“W	*Gallus gallus domesticus*	Vigueras (1934)	RPC
	French Guyana	Unknown	Unknown	53°23′07“N	65°39′25“W	Unknown	Santos-Dias (1958)	RPC
	Mexico	Baja California	Santa Águeda	27°15′36”N	112°21′00”W	Wild dove	Hoffmann (1962)	RPC
		Chiapas	Tapacula	14°54′19”N	92°15′41”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Chichuahua	Chichuahua	28°37′34”N	106°03′44”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Ciudad de Juaréz	31°40′48”N	106°24′55”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Coahuila	San José de Zaragoza	25°07′03”N	103°21′28”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Distrito Federal	Unknown	21°50′14“N	110°18′27“W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Durango	Graceros	23°49′34”N	104°02′38”W	*Gallus gallus domesticus*	Castillo-Martínez et al. (2016)	RPC
			Jalisco	23°59′08”N	104°43′05”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Guanajuato	Guanajuato	21°01′07”N	101°15′36”W	*Zenaida macroura*	Hoffmann (1962)	RPC
		Hidalgo	Unknown	23°18′08“N	110°02′56“W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Mexico	Unknown	27°28′21“N	114°09′31“W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Nuevo León	Monterrey	25°41′12”N	100°18′11”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Puebla	Unknown	21°35′11“N	108°53′29“W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		San Luis Potosi	Tamazunchale	21°15′28”N	98°47′16”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Sinaloa	Cacalotán	23°04′05”N	105°50′28”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Soyatita	24°15′41”N	107°18′28”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Mazatlán	23°14′53”N	106°24′31”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Agua Caliente Grande	26°32′11”N	108°20′50”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Choix	26°42′30”N	108°19′26”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Santa Ana	25°59′24”N	108°25′18”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			San Felipe	26°31′20”N	108°28′27”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			San Joaquín	25°40′02”N	108°01′16”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Sonora	La Dura	28°22′34”N	109°34′05”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Estación Corral	27°37′42”N	109°57′58”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Cumuripa	27°29′31”N	109°56′55”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
			Carrizal	28°26′24”N	109°15′07”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
		Tamaulipas	Tampico	22°16′51”N	97°51′40”W	*Gallus gallus domesticus*	Hoffmann (1962)	RPC
	Nicaragua	Unknown	Unknown	14°24′38“N	94°39′54“W	Domestic bird	Maes et al. (1989)	RPC
	Paraguay	Boquerón	Filadelfia	22°21′08”S	60°02′15”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
	Peru	Lima	Unknown	13°11′39“S	85°16′58“W	*Gallus gallus domesticus*	Need et al. (1991)	RPC

(*continued on next page*)

Table 2 (*continued*)

Species	Country	Administrative division	Locality	Latitud	Longitud	Host	Reference	Observations
		La Libertad	Unknown	08°51′03“S	87°14′37“W	*Gallus gallus domesticus*	Need et al. (1991)	RPC
		Piura	Unknown	05°43′33“S	89°25′44“W	*Gallus gallus domesticus*	Need et al. (1991)	RPC
	Puerto Rico	San Juan	San Juan	18°27′49”N	66°06′13”W	Market place	Capriles and Gaud (1977)	RPC
	United States	California	Grass Valley	39°13′19”N	121°03′39”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			San Joaquin	36°36′36”N	120°11′11”W	*Meleagris galopavus*	Loomis 1953	RPC
		Georgia	Enigma	31°24′45”N	83°19′46”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
		Maryland	Cheverly	38°55′40”N	76°54′55”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			Taneytown	39°39′28”N	77°10′27”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			College Park	38°59′20”N	76°56′09”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			Frederick	39°24′52”N	77°24′51”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			Hebbville	39°20′26”N	76°45′45”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			Baltimore	39°17′77”N	76°36′23”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
		Pensylvania	Hanover	39°48′01”N	76°59′07”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			Hampton	39°55′45”N	77°03′28”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
	Uruguay	Colonia	Unknown	40°25′59“S	64°12′09“W	*Gallus gallus domesticus*	Vogelsang (1928)	RPC
		Paysandú	Unknown	37°40′31“S	63°52′21“W	*Gallus gallus domesticus*	Vogelsang (1928)	RPC
	Venezuela	Aragua	Maracay	10°14′28”N	67°36′24”W	*Gallus gallus domesticus*	Vogelsang and Cordero (1940)	RPC
Argas (P.) miniatus	Antigua and Barbuda	Antigua	Unknown	19°17′56“N	68°47′33“W	Unknown	Hooker (1909)	RPC
	Brazil	Bahia	Queimada Nova	11°01′51”S	42°02′43”W	*Gallus gallus domesticus*	Lorosa et al. (2007)	RPC
			Corguinho	Unknown	Unknown	*Gallus gallus domesticus*	Lorosa et al. (2007)	RPC
			Salvador	12°58′46”S	38°29′33”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Santo Amaro	12°32′54”S	38°42′30”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		Ceará	Fortaleza	03°44′16”S	38°32′22”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Agua Doce	05°19′01”S	39°16′01”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Sobral	03°41′06”S	40°21′15”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Lagoa Grande	03°14′25”S	39°43′26”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		Espirito Santo	Cachoeiro de Itapemirim	20°50′49”S	41°07′19”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Castelo	20°36′15”S	41°12′13”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			São Mateus	18°43′12”S	39°51′16”W	*Gallus gallus domesticus*	Acosta et al. (2016)	CR
			Vitória	20°17′53”S	40°17′50”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		Federal District	Brasilia	15°46′00”S	47°55′00”W	*Gallus gallus domesticus*	Santos et al. (2010)	RPC
				15°37′12”S	47°56′26”W	*Gallus gallus domesticus*	This study	CNC 3409
		Maranhão	São Luis	02°32′14”S	44°16′07”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		Mato Grosso do Sul	Campo Grande	20°28′07”S	54°36′58”W	Unknown	This study	IBSP 1269
		Minas Gerais	Pedro Leopoldo	19°37′15”S	44°02′24”W	*Gallus gallus domesticus*	Ataliba et al. (2007)	RPC
			Belo Horizonte	19°57′04”S	43°55′09”W	*Gallus gallus domesticus*	Kohls et al. (1970), This study	IBSP 9934
			São Roque de Minas	20°15′03”S	46°21′57”W	*Gallus gallus domesticus*	This study	CNC 1154
			Gouveia	18°27′16”S	43°44′27”W	*Gallus gallus domesticus*	Lorosa et al. (2007)	RPC
			Passa Quatro	22°23′21”S	44°58′04”W	*Gallus gallus domesticus*	This study	IBSP 4513
			Juiz de Fora	21°45′50”S	43°20′47”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Guaxupé	21°18′23”S	46°42′38”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Ponte Nova	20°24′48”S	42°53′51”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		Pará	Belém	01°27′10”S	48°28′54”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		Paraiba	Santa Teresinha	07°05′01”S	37°26′34”W	*Gallus gallus domesticus*	This study	CNC 3376, 3382
		Paraná	Apucarana	23°33′04”S	51°27′47”W	*Gallus gallus domesticus*	Arzua et al. (2005)	RPC
			Paranaguá	25°31′05”S	48°31′25”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		Pernambuco	Recife	08°02′08”S	34°53′51”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		Rio de Janeiro	Paraiba do Sul	22°09′36”S	43°17′27”W	*Gallus gallus domesticus*	This study	CNC 878
			Bom Sucesso	22°16′29”S	42°47′43”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			UFRRJ*	22°46′09”S	43°41′14”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
			Santa Cruz do Ibitinema	21°33′43”S	42°17′22”W	*Gallus gallus domesticus*	This study	CNC 2576
			Niterói	22°53′14”S	43°06′53”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Rio de Janeiro	22°54′30”S	43°10′24”W	*Gallus gallus domesticus*	This study	IBSP 731, 803
			Petrópolis	22°30′40”S	43°10′37”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Barra Mansa	22°32′29”S	44°10′34”W	*Gallus gallus domesticus*	Aragão (1936)	RPC

(*continued on next page*)

Table 2 (*continued*)

Species	Country	Administrative division	Locality	Latitud	Longitud	Host	Reference	Observations
			Barra do Piraí	22°28′05”S	43°49′35”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Campos	21°45′40”S	41°19′13”W	*Gallus gallus domesticus*	Rohr (1909)	RPC
			Cantagalo	21°58′49”S	42°21′46”W	*Gallus gallus domesticus*	Rohr (1909)	RPC
			Três Rios	22°06′59”S	43°12′40”W	*Gallus gallus domesticus*	Lisbôa et al. (2009)	RPC
		Rio Grande do Sul	Barra do Riveiro	30°17′50”S	51°18′17”W	*Gallus gallus domesticus*	Evans et al. (2000)	CR
			São Liuz Gonzaga	28°24′23”S	54°57′32”W	*Gallus gallus domesticus*	Evans et al. (2000)	CR
			Ijuí	28°23′18”S	53°55′03”W	*Gallus gallus domesticus*	Evans et al. (2000)	CR
			Santo Angelo	28°17′49”S	54°16′02”W	*Gallus gallus domesticus*	Evans et al. (2000)	CR
			Alegrete	29°47′11”S	55°47′43”W	*Gallus gallus domesticus*	Evans et al. (2000)	CR
			Porto Alegre	30°01′43”S	51°11′33”W	*Gallus gallus domesticus*	Evans et al. (2000)	CR
			Santa Maria	29°41′26”S	53°48′50”W	*Gallus gallus domesticus*	Evans et al. (2000)	CR
			Sarandí	27°56′47”S	52°55′01”W	*Gallus gallus domesticus*	Evans et al. (2000)	CR
		Santa Catarina	Florianópolis	27°35′41”S	48°32′48”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
		São Paulo	São Paulo	23°26′23”S	46°40′59”W	*Gallus gallus domesticus*	This study	IBSP 937
				23°26′23”S	46°40′59”W	*Gallus gallus domesticus*	Nóbrega et al. (1947)	RPC
				23°26′23”S	46°40′59”W	*Gallus gallus domesticus*	Keirans (1984)	RPC
			Campinas	22°54′39”S	47°03′48”W	*Gallus gallus domesticus*	Rohr (1909)	RPC
			Amparo	22°42′30”S	46°46′24”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Baurú	22°19′03”S	49°03′47”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Santos	23°57′46”S	46°19′48”W	*Gallus gallus domesticus*	Aragão (1936)	RPC
			Jaboticabal	21°15′11”S	48°19′28”W	*Gallus gallus domesticus*	This study	IBSP 4317
			Canindé	23°31′38”S	46°37′09”W	*Gallus gallus domesticus*	This study	IBSP 1279
			Piracicaba	22°44′14”S	47°39′20”W	*Gallus gallus domesticus*	This study	IBSP 9251
			Butantan	23°34′04”S	46°43′12”W	*Gallus gallus domesticus*	This study	IBSP 1746
			Rancharia	22°13′38”S	50°53′44”W	*Gallus gallus domesticus*	This study	IBSP 4214
			Santa Caleana	Unknown	Unknown	*Gallus gallus domesticus*	This study	IBSP 1267
			Unknown	25°24′33“S	54°14′00“W	*Gallus gallus domesticus*	This study	IBSP 5954
	Colombia	Magdalena	Santa Marta	11°13′43”N	74°11′36”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
	Cuba	Camagüey	Camagüey	21°23′10”N	77°54′34”W	*Gallus gallus domesticus*	This study	CZACC 10704
		Pinar del Río	San Cristóbal	22°42′56”N	83°03′03”W	*Gallopavo meleagris*	De La Cruz (1974)	CZACC 10734
	Guyana	Demerara	Unknown	07°22′05“N	65°05′34“W	Unknown	Koch (1844)	CR
	Jamaica	Unknown	Unknown	20°32′57“N	86°04′16“W	*Gallus gallus domesticus*	Keirans (1984)	RPC
	Martinica	Unknown	Unknown	16°29′31“N	67°54′53“W	Unknown	Hooker (1909)	RPC
	Panama	Panama	Panama City	09°04′02”N	79°31′11”W	*Gallus gallus domesticus*	Fairchild et al. (1966)	RPC
		Colón	Colón	09°19′59”N	79°53′56”W	*Gallus gallus domesticus*	Dunn (1923)	RPC
		Canal Zone	Unknown	10°08′13“N	87°46′58“W	*Gallus gallus domesticus*	Dunn (1923)	RPC
	Puerto Rico	Peñuelas	Peñuelas	18°03′21”N	66°43′19”W	*Gallus gallus domesticus*	Capriles and Gaud (1977)	RPC
	Trinidad and Tobago	Trinidad	Gasparee Island	10°39′57”N	61°39′18”W	*Gallus gallus domesticus*	Kohls et al. (1970)	CR
	United States	Florida	Miami	25°45′48”N	80°11′56”W	Unknown	Keirans (1984)	RPC
	Venezuela	Unknown	Unknown	07°58′14“N	73°37′24“W	*Gallus gallus domesticus*	Vogelsang and Dias (1953)	RPC

*Campus of the “Universidade Federal Rural do Rio de Janeiro”, Rio de Janeiro State.

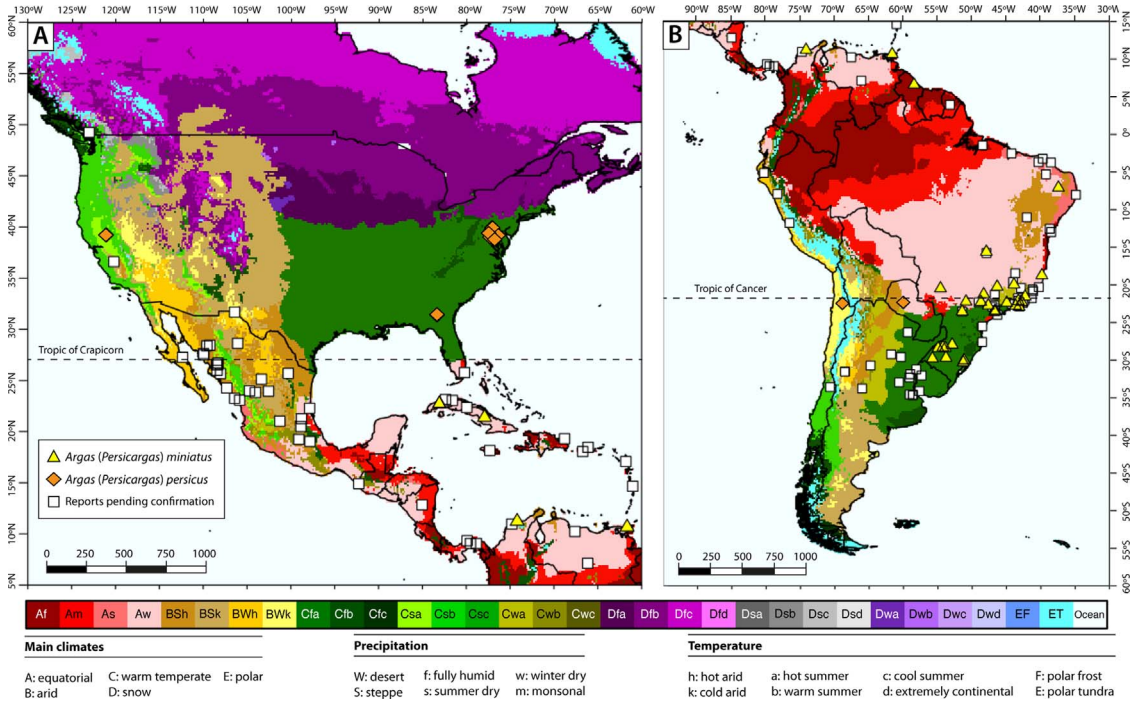

Fig. 5. Distributional maps of *Argas* (*Persicargas*) *miniatus* and *Argas* (*Persicargas*) *persicus* plotted over the Köppen-Geiger climate classification of America (Kottek et al., 2006). (A) Map of North and Central America. (B) Map of South America.

(Marchoux and Salimbeni, 1903; Nóbrega and Reis, 1947; Loomis, 1953; Ataliba et al., 2007; Lisbôa et al., 2009), which is transmitted by these group of ticks. However, early reports of both species for America are unclear and it is difficult to assign an identity without a reexamination of the collected material. For this reason the majority of the records for *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* should be considered as provisory or pending confirmation.

4.3.1. North America

In Canada, four nymphs of *A.* (*P.*) *miniatus* were identified by Hearle (1938) from specimens collected in a golden-crowned sparrow (*Zonotrichia atricapilla*) at Vancouver. Cooley and Kohls (1944) and Gregson (1956) subsequently referred to this record as *A.* (*P.*) *persicus*, which to date, corresponds to the sole report of this species for this country and to the northernmost record for America.

In the United States, reports of *A.* (*P.*) *persicus* from Arizona, Florida, Iowa, Nevada, New Mexico, Texas and Utah states, summarized by Bishopp (1941) and Cooley and Kohls (1944), were subsequently identified either as *A.* (*P.*) *radiatus* or *A.* (*P.*) *sanchezi* by Kohls et al. (1970). These same authors posed the possibility that *A.* (*P.*) *miniatus* could have been introduced into the United States; however the inadequate state of the material available for examination precluded an accurate species determination (Kohls et al., 1970). On the other hand, Hoogstraal (1985) still assumed that this species does occur in "small foci" in southeastern regions of the United States.

Hoffmann (1962) listed the distribution of *A.* (*P.*) *persicus* for Mexico, naming several states along the country. Remarkably, some of the reports had a common distribution with the geographical range of *A.* (*P.*) *radiatus* and *A.* (*P.*) *sanchezi* (Baja California, Durango, Guanajuato, and Tamaulipas) proposed by Kohls et al. (1970). Moreover, according to Hoffmann (1962), *A.* (*P.*) *persicus* has been collected from the morning dove *Zenaida macroura* (L. 1758), which constitutes the type host of *A.* (*P.*) *sanchezi* (Kohls et al., 1970). While it is highly possible that *A.* (*P.*) *persicus* occurs in Mexico, historical confusion with other *Argas* species precludes an accurate distribution for this country (Hoffmann and López-Campos, 2000).

4.3.2. Central America and Caribe

Panamanian reports of *A.* (*P.*) *persicus* (Fairchild et al., 1966) were subsequently questioned by Kohls et al. (1970), noting that these specimens could have been misidentified, and rather correspond to *A.* (*P.*) *miniatus*. In fact Kohls et al. (1970) included immature and mature specimens of unknown host and locality collected in Panama when they redescribed *A.* (*P.*) *miniatus*. Early reports of Dunn (1923) already included this tick as parasites of domestic chicken in this country, which supports the presence of *A.* (*P.*) *miniatus* and not *A.* (*P.*) *persicus* in Panama.

The first report of *A.* (*P.*) *miniatus* in Cuba was presented by Hooker (1909) in a review of the geographical distribution of ticks from America. Since then, the presence of this tick was not questioned for the island. However, Kohls et al. (1970) excluded Cuba within its distribution, presumably because they did not examine any available material (De La Cruz, 1976). On the other hand, after the examination of soft ticks collected in a poultry farm from Camagüey (CZACC 10704), De La Cruz (1976) stated that *A.* (*P.*) *persicus* was also established in Cuba. In the current study, we were able to morphologically examine one male and three nymphs of this original allotment, and found that these ticks corresponded to *A.* (*P.*) *miniatus*. Moreover, we confirmed the presence of this tick in Cuba by the examination of an additional *Argas* allotment from San Cristóbal (CZACC 10734).

In other Caribbean islands where both species of soft tick have been reported (Antigua and Puerto Rico), new assessments must be undertaken, either by the collection of new material, or by the reexamination of deposited specimens in order to clarify the identity of these ticks. In the same way, records of *A.* (*P.*) *persicus* from Nicaragua documented by Maes et al. (1989) should be considered as doubtful.

4.3.3. South America

The first report of *A. (P.) miniatus* for Brazil was made in 1903 by Marchoux and Salimbeni (1903), as part of research with *B. anserina*. Subsequent literature referred to this tick as a synonym of *A. (P.) persicus* in America (Nuttall et al., 1908), and particularly in Brazil, *A. (P.) miniatus* was considered to be a morphological variety named as *A.* (*P.*) *persicus* var. *dissimile* (Aragão, 1936). In the present study we examined

the totality of Brazilian allotments of *Argas* ticks deposited in the IBSP, and concluded that all the specimens corresponded to *A. (P.) miniatus*. With this, we confirm that this species has a wide distribution in this country. Moreover, it is highly possible that the rest of unconfirmed reports might also correspond to the same species.

While the presence of *A.* (*P.*) *miniatus* has not been confirmed for Argentina, Guglielmone and Nava (2005) considered that reports of *A.* (*P.*) *persicus* published by early and contemporary researchers (o, 1935, 1938; o, 1935, 1938; Boero, 1945; Ivancovich and Luciani, 1992) would rather correspond to *A. (P.) miniatus*. Remarkably, Nava et al. (2004) confirmed the presence of *A.* (*P.*) *persicus* in Argentina through a morphological analysis of one female of unknown host and locality. This fact still leaves as uncertain the occurrence of *A.* (*P.*) *miniatus* in this country, and raises the possibility that previous reports of *A.* (*P.*) *persicus* might be valid.

In Chile, the reports of *A.* (*P.*) *persicus* made by Lahille (1915) and Porter (1928), with material collected in Calama, were subsequently considered synonyms of *A.* (*A.*) *neghmei* by Kohls and Hoogstraal (1961). Here, combining a morphological analysis of larvae with a molecular characterization of these and other postlarval stages, we confirm the presence of *A.* (*P.*) *persicus* for the first time in this country. As our collections were also from Calama, it implicates that this soft tick might occur in sympatry with *A.* (*A.*) *neghmei*. Since Lahille (1915) also reported an *A.* (*P.*) *persicus* variety from San Bernardo in the Metropolitan Region, the distribution of this tick in Chile might be underestimated.

Although Vogelsang (1928) reported the presence of *A.* (*P.*) *persicus* for Uruguay, it is probable that *A.* (*P.*) *miniatus* also occurs in this country (Venzal et al., 2003). In the same way, while *A.* (*P.*) *persicus* occurs in Paraguay (Kohls et al., 1970) the presence of *A.* (*P.*) *miniatus* should not be discarded (Nava et al., 2007).

4.4. Distribution according to Köppen-Geiger climate classification

When contrasted to the Köppen-Geiger climate classification of America, confirmed reports for *A.* (*P.*) *miniatus* are almost exclusively distributed within an equatorial climate regime well delimited by both tropic lines. Conversely, the distribution of *A.* (*P.*) *persicus* excludes equatorial climates, and rather overlaps with arid climates, which in particular, correspond to an ecologically constant trait among its world distribution (Hoogstraal, 1956, 1985; Petney et al., 2004). Apart of this apparently dual distribution according to equatorial and arid climate regimes, both ticks share distributional ranges within warm temperate climates: *A.* (*P.*) *miniatus* in South America, and *A.* (*P.*) *persicus* in North America. On the other hand, more than a half (69%) of the reports are yet to be confirmed, and they are located in all three above stated climates. Considering this distributional scenario, unless reports pending confirmation are properly determined, any major conclusion on the distribution of both soft ticks in accordance to climates of the American continent will be rather speculative.

Conflict of interest

The authors declare no financial or personal conflicts of interest that could bias the study.

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