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Taxonomic Status of *Ixodes didelphidis* (Acari: Ixodidae)

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ABSTRACT *Ixodes didelphidis* Fonseca & Aragão was described in Brazil in 1952 as a new tick species that differed from *Ixodes loricatus* Neumann by the spiracular plate pattern. We have reared four tick colonies from different geographic areas in the laboratory that were started from single engorged females originally identified as *I. didelphidis* (BMG colony) and *I. loricatus* (CSP, PSP, and TRJ colonies). We analyzed the spiracular plate morphology of F_1 adult ticks from each tick colony, compared their biological data, and performed a molecular analysis of the second internal transcribed rDNA spacer (ITS2) to test the validity of the species *I. didelphidis.* The spiracular plate analysis of laboratory F_1 adult ticks yielded from single females from the four colonies showed variations that invalidate morphological parameters for differentiation of *I. loricatus* and *I. didelphidis.* Biological data of the BMG, CSP, and TRJ colonies were similar. The biology of the PSP colony was not evaluated. The ITS2 sequence variations observed between the tick colonies ranged from 1.3 to 4.9%, and the similarity tree constructed by the neighbor-joining method with nucleotide distances showed that the distances between the samples were similar to what is expected for intraspecific variations found in other ticks species. The morphological and biological results, in conjunction with the ITS2 analysis, supported the conspecificity of *I. loricatus* and *I. didelphidis.*

RESUMO *Ixodes didelphidis* Fonseca e Aragão foi descrito no Brazil em 1952 como uma nova espécie de carrapato que se diferenciava de *Ixodes loricatus* Neumann através do padrão da placa espiracular. Nós criamos em laboratório quatro colônias de carrapatos de diferentes áreas geográficas, iniciadas a partir de fêmeas ingurgitadas originalmente identificadas como *I. didelphidis* (colônia BMG) e *I. loricatus* (colônias CSP, PSP, e TRJ). Nós analisamos a morfologia da placa espiracular dos adultos F_1 de cada colônia, comparamos seus dados biológicos e fizemos uma análise molecular do segundo espaço transcrito interno do rDNA (ITS2) para verificar a validade da espécie *I. didelphidis.* A análise da placa espiracular de adultos provenientes de uma mesma fêmea de cada uma das colônias demonstrou resultados controversos que invalidam os parâmetros morfológicos publicados para diferenciação de *I. loricatus* e *I. didelphidis.* Os dados biológicos das colônias BMG, CSP, e TRJ foram similares. As variações nas seqüências do ITS2 entre as colônias de carrapatos (amplitude: 1,3–4,9%) e a árvore de similaridade com as distâncias de nucleotídeos mostraram que as distâncias entre as amostras foram similares ao esperado para variações intra-específicas encontradas em outras espécies de carrapatos. Os resultados morfológicos, biológicos e moleculares fornecem evidências para considerarmos *I. loricatus* e *I. didelphidis* como a mesma espécie.

KEY WORDS *Ixodes loricatus*, *Ixodes didelphidis*, spiracular plate, biology, second internal transcribed spacer

During the last two decades, several studies have been conducted on the biology, epidemiology, and taxonomic status of various members of the genus *Ixodes*, as well as their interaction with various pathogens. Recent investigations determined the capability of *Ixodes* ticks for transmitting *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner sensu lato genospecies to humans or domestic and wild animals, indicating the tick potential for maintaining enzootic foci of borreliosis (Gray 1998, Oliver 1999). More than 35 species of *Ixodes* have been reported in the Neotropical zoogeographic region, but this genus is one of the most difficult to distinguish taxonomically and there are still several doubtful species throughout the world (Clifford et al. 1973).

We followed the protocol, which agrees with Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation (COBEA), and was approved by the Biomedical Sciences Institute/USP-Ethical Committee for Animal Research (CEEA). Permits and approvals are on file in the office of T.T.S.S.

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Fonseca and Aragão (1952) described a tick collected from opossums in Brazil, which they called *Ixodes didelphidis* Fonseca & Aragão, a species closely related to *Ixodes (Ixodes) loricatus* Neumann. This later species is a common ectoparasite of opossums in central-southern Brazil, and has been reported from Mexico to Argentina (Cooley and Kohls 1945). According to Barros-Battesti and Knysak (1999), the occurrence of *I. didelphidis* is confirmed in the southern, southeast, and central-west regions of Brazil. Fonseca and Aragão (1952) used the shape of the spiracular plate and the number of goblets to differentiate these two species. In the original description of *I. didelphidis*, the authors presented figures showing that the male and female spiracular plates of *I. didelphidis* were larger and had a higher number of goblet lines (GL) than the respective sexes of *I. loricatus.* Moreover, Aragão and Fonseca (1961) presented a taxonomic key for Brazilian ticks in which they indicated that in *I. didelphidis* males, the spiracular plate was elliptical and composed of a minimum of 8 GL between the macula and the superior border of the spiracular plate, contrasting to *I. loricatus* in which the spiracular plate was oval and composed by a maximum of 5 GL between the macula and the superior border. According to this same key, *I. didelphidis* females have an elliptical spiracular plate composed of 8 GL between the macula and the largest area of the spiracular plate, whereas *I. loricatus* females have a rounded spiracular plate composed of 5 GL between the macula and the largest area of the spiracular plate.

We have examined several specimens that we identified as *I. loricatus* using other taxomomic keys (Cooley and Kohls 1945, Fairchild et al. 1966) that do not include *I. didelphidis* as a valid species. Using the key of Aragão and Fonseca (1961), some of these specimens were identified as *I. didelphidis* but others as *I. loricatus.* Similar observations were recorded on F_1 adults from four tick colonies reared from different areas. A single female yielded some F_1 adults that were identified as *I. loricatus*, whereas others keyed to *I. didelphidis* in Aragão and Fonseca (1961). Because of these findings, we analyzed the spiracular plates of adult ticks, biological data of different populations and performed a molecular analysis of the second internal transcribed rDNA spacer (ITS2) to test the validity of the species *I. didelphidis.*

Materials and Methods

Ticks. Engorged females collected from opossums in four geographic areas of Brazil were identified according to Aragão and Fonseca (1961) and used to start four independent colonies in the laboratory. The four colonies were labeled according to their geographic origin as follows: Colony BMG; one female collected from *Didelphis albiventris* at Belo Horizonte (19° 49′ S, 43° 57′ W), State of Minas Gerais (MG), identified as *I.* (*I.*) *didelphidis.* Colony CSP; one female collected from *Didelphis marsupialis* at Cotia (23° 36′ S; 46° 55′ W), state of São Paulo (SP), identified as *I.* (*I.*) *loricatus.* Colony PSP; one female collected from *Didelphis* sp at Pirassununga (21° 59′ S, 47° 25′ W), SP, identified as *I.* (*I.*) *loricatus.* Colony TRJ; one female collected from *Didelphis* sp at Teresópolis (22° 24′ S, 42° 57′ W), State of Rio de Janeiro (RJ), identified as *I.* (*I.*) *loricatus.*

After rearing one or two generations of each colony in the laboratory, at least 20 males and 20 females from each colony were identified using the key of Aragão and Fonseca (1961). Additionally, adult specimens from the four colonies were randomly selected for the molecular analysis described bellow.

Biological Comparison. Life-cycle data of the BMG and CSP colonies obtained during laboratory rearing were published by Schumaker et al. (2000) and were used for comparison with the TRJ colony data. Biological data relative to the life-cycle of the PSP colony was not recorded. In the current study, we report the life-cycle data of two generations of each stage of the TRJ colony reared under laboratory conditions. Naive Wistar rats, *Rattus norvegicus* Berkenhout, were used as hosts for the immature stages, and field caught white-belly opossums (*D. albiventris*) were used to feed adult ticks. Throughout this study, ticks were reared at 27°C and 95–98% RH in an incubation oven maintained under total darkness, except when ticks were feeding on the host. All infestation procedures were performed as described by Schumaker et al. (2000) for the BMG and CSP colony-life-cycle study.

From two generations of the TRJ tick colony, 2,600 larvae and 150 nymphs were placed on nine (average 288 ticks per host) and five (30 nymphs per host) *R. norvegicus.* Fifteen pairs of adult ticks were put on three opossums (five pairs per host). After the larvae hatched or the nymphs and adults ecdysed, the newly emerged ticks were held for specified prefeeding periods of ≈50, 30, and 40 d, respectively, before being placed on a host (Schumaker et al. 2000).

DNA Extraction, Amplification and Sequencing. F_1 adult male ticks from the colonies BMG (three ticks), CSP (two ticks), PSP (two ticks), and TRJ (two ticks) were individually used for polymerase chain reaction (PCR) amplification. The BMG, CSP, and PSP males were of the *I. didelphidis* type, whereas the TRJ males were of the *I. loricatus* type. Genomic DNA was extracted according to Zahler et al. (1995) with some modifications. Live individual ticks were killed in liquid nitrogen and homogenized in 400 μl TET buffer (50 mM Tris-HCl, 25 mM EDTA, 100 mM NaCl, 0.4% Triton X-100) and 10 μl (20 mg/ml) proteinase K. After incubation at 56°C for 2 h, the DNA was extracted by shaking in 200 μl PCI (phenol: chloroform: isoamylic alcohol 25:24:1) and precipitated by cold ethanol overnight. After centrifugation (12,000 × *g* for 5 min), the pellet was resuspended in 35 μl of TE (10 mM Tris, 1 mM EDTA) and used for PCR.

PCR for specific amplification of the ITS2 was conducted according to Zahler et al. (1995) using the primer (1) 5′CCATCGATGTGAAYTGCAGGACA3′ in the 5.8S rDNA gene (Zahler et al. 1995) and the primer (2) 5′GTGAATTCTATGCTTAAATTCAG GGGGT3′ in the 28S rDNA gene (McLain et al. 1995). The PCR reaction was conducted for 35 cycles (95°C

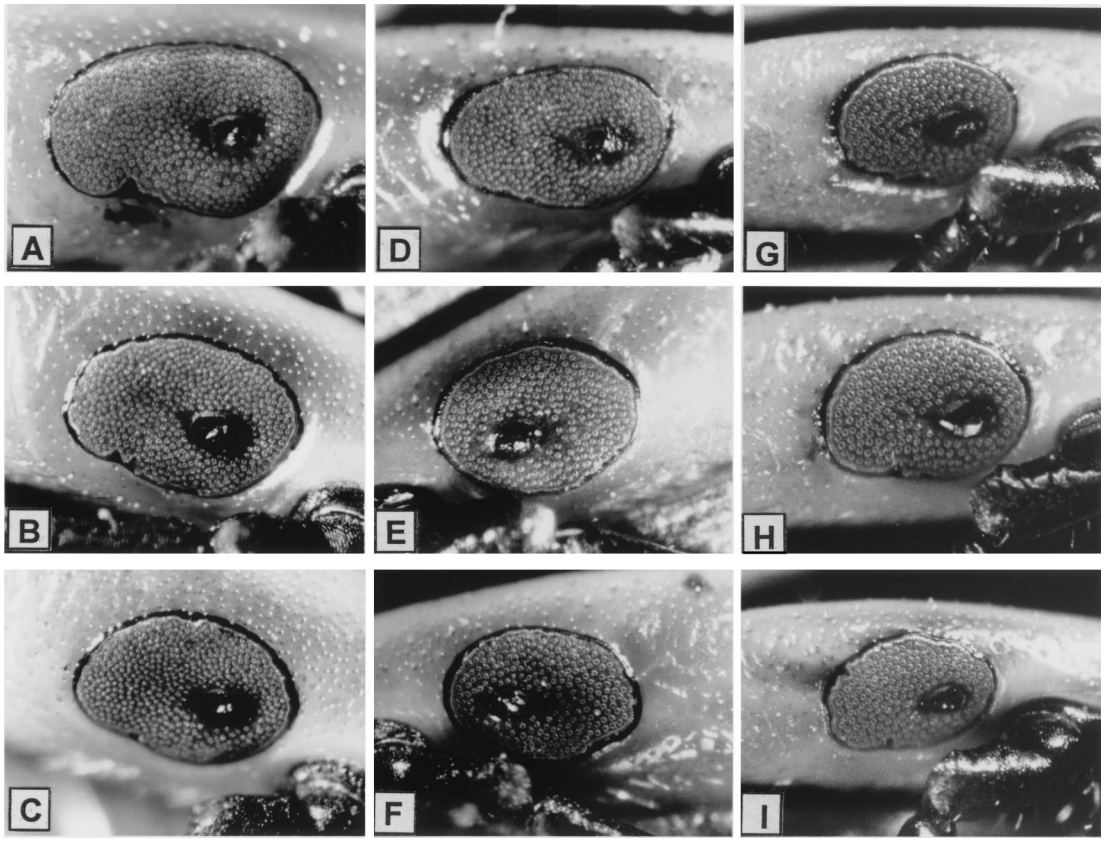

Fig. 1. Spiracular plates of *I. loricatus* adult males from three populations: BMG, CSP, and TRJ. (A, B, and C) Three individuals from BMG. (D, E, and F) Three individuals from CSP. (G, H, and I) Three individuals from TRJ. All photographs were taken in an stereoscope microscope under 70× lens.

for 45 min, 55°C for 1 min, and 72°C for 1.5 min) and the products (≈800 bp) were visualized by ethidium bromide staining after electrophoresis in a 0.8% agarose gel. DNA fragments obtained after PCR were ligated into pGEM-T easy vector plasmid (Promega, Madison, WI), and introduced into competent *Escherichia coli* DH5α cells. At least two clones of each individual were sequenced in an automatic sequencer using ABI Prism dGTP BigDye Terminator Ready Reaction Kit (Perkin-Elmer, Foster City, CA).

Sequence Analysis. The sequence data were aligned using the CLUSTAL W (1.60) program (Thompson et al. 1994). Aligned sequences were examined using the program MEGA (Molecular Evolutionary Genetics Analysis version 1.01) (Kumar et al. 1993) and a similarity matrix was constructed. Relationships between specimens were assessed by the unweighted pairgroup method with arithmetic means, and by neighbor-joining method. A similarity tree constructed by the neighbor-joining (NJ) method with nucleotide distances (p-distance) was used to compare the ITS2 sequences. The ITS2 sequences of *Ixodes* (*Ixodes*) *scapularis* (Say) (L22273–1), *Ixodes* (*Ixodes*) *pacificus* (Cooley & Kohls) (L22279–1), and *Ixodes* (*Ixodes*) *ricinus* (L.) (D88884–1) ticks were obtained from GenBank and used as outgroups in the similarity tree. The nucleotide sequences of the ITS2 region of *Ixodes* ticks reported in this article have been submitted to GenBank/EMBL Databases with the following accession numbers: BMG (AF327341), CSP1 (AF327339), CSP2 (AF327340), PSP1 (AF327343), PSP2 (AF327344), TRJ (AF327342).

Results

Spiracular Plates. The spiracular plate shape did not show a defined pattern within the adult ticks of a given colony, being elliptical in some specimens and oval or rounded in others (Figs. 1 and 2). The identification of F_1 adults in the taxonomic key of Aragão and Fonseca (1961) was based on the number of GL in the spiracular plates.

Colony BMG. All males were identified as *I. didelphidis*, showing a minimum of 8 GL between the macula and the superior border of the spiracular plate (Fig. 1 A–C). The majority of females were identified as *I. didelphidis*, showing ≈9 GL between the macula and the largest area of the spiracular plate (Fig. 2C), whereas a few specimens did not fit in the taxonomic key of Aragão and Fonseca (1961) as they had >5 GL but <9 GL (Fig. 2 A and B).

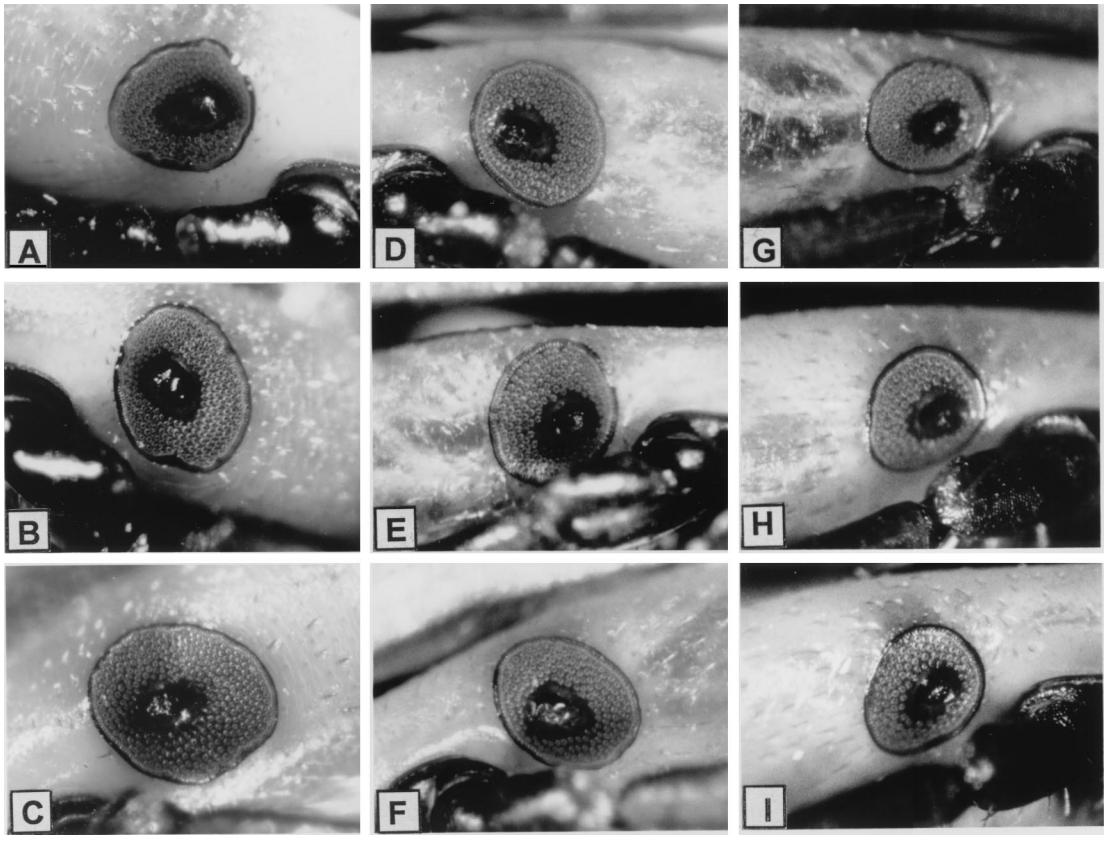

Fig. 2. Spiracular plates of *I. loricatus* adult females from three populations: BMG, CSP and TRJ. (A, B, and C) Three individuals from BMG. (D, E, and F) Three individuals from CSP. (G, H, and I) Three individuals from TRJ. All photographs were taken in a stereoscope microscope under 70× lens.

Colony CSP. All males were identified as *I. didelphidis*, showing ≈8 GL between the macula and the superior border of the spiracular plate (Fig. 1 D–F). The females were more similar to the *I. loricatus* type, because they presented 5 or 6 GL (Fig.2 D–F). Ticks from the colony PSP followed a similar pattern to the CSP ticks.

Colony TRJ. The great majority of males were identified as *I. loricatus*, showing a maximum of 5 GL (Fig. 1 G and I), whereas a few specimens did not fit the key because they presented >5 GL and <8 GL (Fig. 1H). All females were identified as *I. loricatus*, as they presented 5 GL (Fig. 2 G–I).

Life Cycle of the TRJ Colony. The biological and developmental data, excluding the prefeeding periods, obtained from two separate rearing generations for the TRJ colony are presented in Table 1. A mean period of 220 d (including prefeeding periods) was

Table 1. Biological data for *I. loricatus* from the TRJ colony under laboratory conditions

Factor	Tick stage		
	Larvae	Nymphs	Females
Host	*R. norvegicus*	*R. norvegicus*	*D. albiventris*
No. exposed	2,600	150	15
No. recovered (%)	330 (12.6)	70 (46.6)	10 (66.6)
Feeding period (d)[a]	6.8 ± 1.0 (5–10)	6.5 ± 0.8 (5–8)	10.2 ± 1.1 (9–12)
No. molted or oviposited (%)	211 (8.1)	58 (38.6)	6 (60.0)
Premolt or preoviposition period (d)[a]	9.8 ± 1.9 (8–17)	20.4 ± 1.1 (18–24)	6.3 ± 1.0 (5–8)
Weight of engorged female (mg)[a]	—	—	335.8 ± 61.8 (216–424)
Weight of deposited eggs (mg)[a]	—	—	115.6 ± 48.1 (50–185)
Egg incubation period (d)[a]	—	—	39.6 ± 9.3 (31–50)
Egg hatch (%)[a]	—	—	42.7 ± 37.5 (0–99)
Egg production efficiency (EPE)[a]	—	—	34.0 ± 15.9 (13.1–56.1)

[a] Mean ± SD (range).

required to complete an entire cycle from adult female to the next generation of females.

ITS2 Sequence. The complete sequences of ITS2 and flanking regions and the alignment between them are shown in Fig. 3. These include 96 bp of the 3′ end of 5.8S and 51 bp of the 5′ end of 28S. The limits of the ITS2 were defined by similarity with sequences from other tick species (Wesson et al. 1993, Barker 1998, Fukunaga et al. 2000). Thus, the observed number of nucleotides in the ITS2 sequence ranged from 813 in the TRJ colony to 819 in the PSP colony. The three samples from the BMG colony had identical ITS2 sequences (816 bp) as did the two samples of TRJ colony (813 bp). For this reason, only one individual sequence from each of these two colonies is shown in the alignment. The two samples from the CSP colony had the same sequence lengths (815 bp) but the nucleotide alignment was not identical. The two individuals from the PSP colony had ITS2 sequences of different lengths, one was 819 bp long, and the other was only 816 bp. No ITS2 clonal variation was found in all analyzed individuals.

The alignment of these regions showed that the inter-colony variation between the ITS2 sequences varied from 1.3 to 4.9%. Except for the insertion/deletion of three nucleotides at position 660 of the BMG sequence, all other differences (insertions/deletions, transitions or transversions) found among the sequences were at one or two nucleotide sites. Within the tick colonies, variations in the sequences were 0.0% in the BMG and TRJ colonies, 0.9% (7 sites) in the PSP colony, and 1% (8 sites) in the CSP colony (Table 2).

The BMG colony was the most divergent, because the nucleotide variations occurred in 31–40 of the 816 sites (3.8–4.9%) when compared with the other colonies (Table 2). When the TRJ colony was compared with other colonies, the nucleotide variation was in 16–40 of the 814 sites (1.9–4.9%). One of the individuals of the PSP colony had divergences in 12–36 of 819 sites (1.5–4.4%), and the other had 11–33 differences in 816 sites (1.3–4.0%). When the two individuals from the CSP colony were compared with individuals from other colonies nucleotide variations were found in 11–33 of the 815 sites (1.3–4.0%) for one individual and 16–31 of the 815 sites (1.9–3.8%) for the other individual.

The unweighted pair-group method with arithmetic average (tree not showed) and the neighbor-joining (Fig. 4) tree showed the same pattern. All ticks were clustered according to geographic proximity of sites of origin. Thus, the CSP and PSP ticks could represent the São Paulo State clade. The TRJ ticks represented the Rio de Janeiro clade, whereas the BMG ticks formed the Minas Gerais State clade. We also compared the sequences obtained in this study with other ITS2 sequences from three distinct species of *Ixodes*, used as outgroups. The degrees of divergence seen in Table 2, as well as the distances shown in the tree, indicate clearly that the sequences obtained from the ticks analyzed in this study grouped together as expected for individuals or populations of the same species.

Discussion

The spiracular plate analysis of adult ticks obtained from individual single females from four tick populations showed some conflicting results that invalidate the taxonomic key proposed by Aragão and Fonseca (1961) for differentiation of *I. loricatus* and *I. didelphidis*, because some specimens did not fit in the key parameters. Additionally, some colonies (CSP and PSP) yielded males of the *I. didelphidis* type and sibling females of the *I. loricatus* type.

Schumaker et al. (2000) reported several biological parameters for the BMG and CSP ticks under similar laboratory conditions to the present biological study on the TRJ ticks. The mean feeding periods (range, in parentheses) of BMG larvae, nymphs and adult females were 6.4 d (5–9), 6.3 d (5–9), and 11.4 d (9–14), respectively; whereas the feeding values of CSP larvae, nymphs and females were 6.8 d (5–9), 7.4 d (5–10), and 8.7 d (6–12), respectively. The mean premolt periods of BMG and CSP larvae were 10.2 d (7–22) and 10.6 d (8–19), respectively, and the mean premolt periods of BMG and CSP nymphs were 22.3 d (21–26) and 20.6 d (18–26), respectively. The mean preoviposition periods of BMG and CSP engorged females were 6.3 d (4–8) and 5.6 d (5–7), respectively. The BMG engorged females weighed an average of 327.4 mg (228–409), their egg masses had a mean weight of 135.0 mg (50–214), the egg mean incubation period was 38.8 d (34–42), and the mean egg production efficiency (EPE) was 40.8 (23.6–54.8). The CSP engorged females weighed an average of 313.1 mg (256–389), their egg masses had a mean weight of 138.4 mg (84–177), the egg mean incubation period was 38.4 d (35–42), and the mean egg production efficiency (EPE) was 44.0 (32.2–53.4). Mean egg hatches were 56.8% (5–90) and 42.9% (10–80) for the BMG and CSP colonies, respectively. These biological data of BMG and CSP ticks were very similar to those recorded for the TRJ ticks in Table 1.

Because of these results, we performed a molecular analysis of the second internal transcribed rDNA spacer (ITS2) of some individuals of these colonies to test the validity of the species *I. didelphidis.* Analysis of ITS2 regions has been used to solve systematic problems and to study evolutionary relationships among various organisms (Hillis and Dixon 1991) such as ticks and mosquitoes (Wesson et al. 1993, Zahler et al. 1995, Marrelli et al. 1999, Fukunaga et al. 2000).

In this study, the ITS2 sequence sizes of nine individual ticks representing four populations varied from 813–819 bp. However, within the colonies, individual sequences of sibling ticks tended to have the same length as only two individuals from the PSP colony had the sequences of different lengths (Table 2). Fukunaga et al. (2000) reported smaller sizes for the ITS2 sequences of 12 *Ixodes* species from the Old World (range, 702–802). Among Neartic *Ixodes* species, Wesson et al. (1993) reported ITS2 sequences even

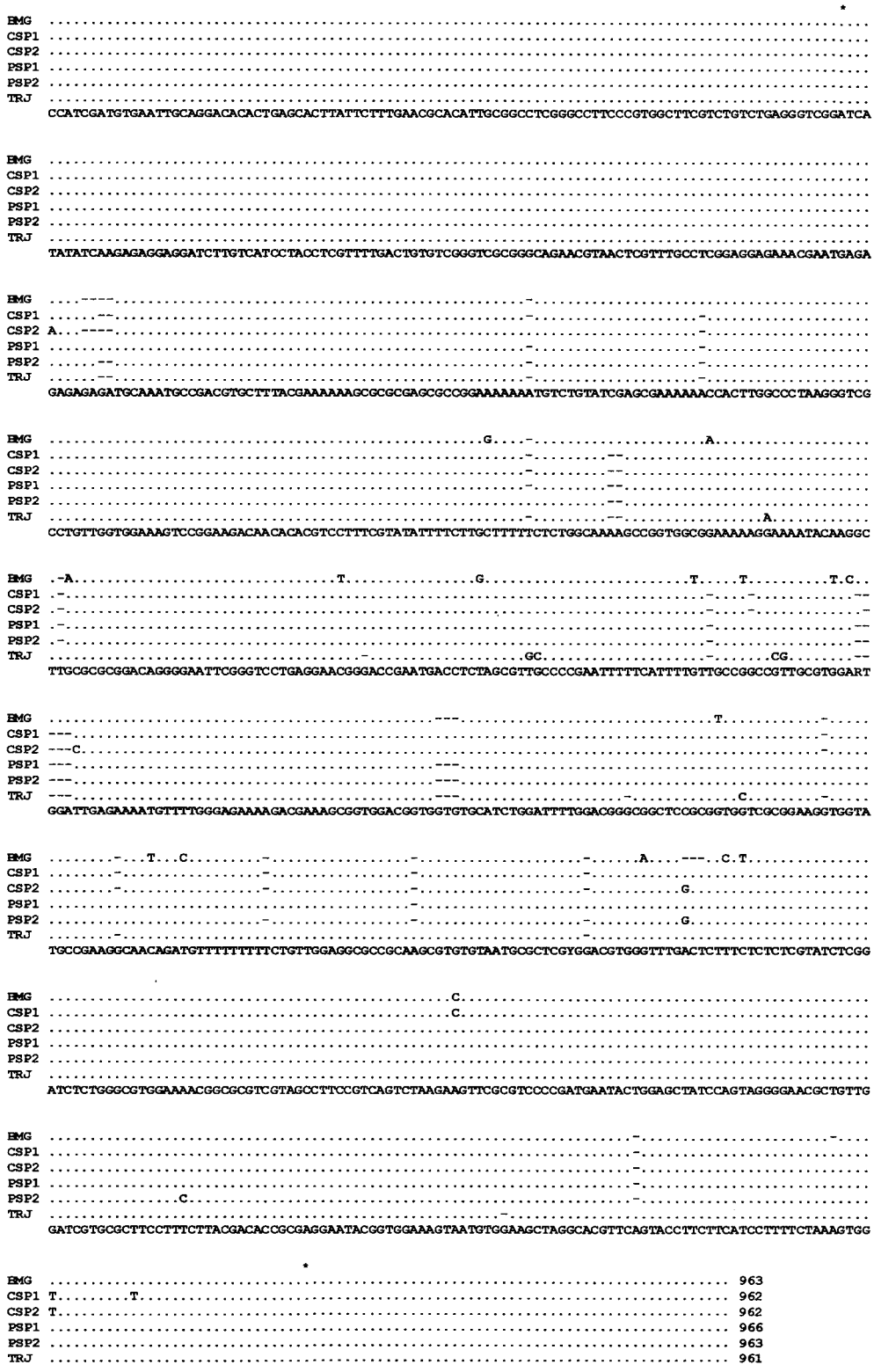

Fig. 3. Alignment of nucleotide sequences (5′ to 3′) of the ITS2 and flanking 5.8S (nucleotides 1–96) and 28S (the 51 end nucleotides) regions of ribosomal DNA of *I. loricatus* (Asterisks indicate the limits of ITS2).

Table 2. Divergence matrix for the ITS2 nucleotide sequences of individual *I. loricatus* ticks from four localities in Brazil compared with outgroup ticks

Ticks	Divergence, %									Outgroups		
	BMG1	BMG2	BMG3	CSP1	CSP2	PSP1	PSP2	TRJ1	TRJ2	*I. sca*	*I. pac*	*I. ric*
BMG1	—	0.0	0.0	4.0	3.8	4.4	4.0	4.9	4.9	56.0	53.9	55.1
BMG2		—	0.0	4.0	3.8	4.4	4.0	4.9	4.9	56.0	53.9	55.1
BMG3			—	4.0	3.8	4.4	4.0	4.9	4.9	56.0	53.9	55.1
CSP1				—	1	1.5	1.3	1.9	1.9	56.1	54.1	55.4
CSP2					—	1.9	2.1	2.5	2.5	56.2	54.1	55.4
PSP1						—	0.9	2.0	2.0	56.6	54.6	55.4
PSP2							—	2.0	2.0	56.8	54.5	55.6
TRJ1								—	0.0	56.8	54.4	55.4
TRJ2									—	56.8	54.4	55.4
I. sca										—	21.3	26.6
I. pac											—	24.0
I. ric												—

BMG: Belo Horizonte, MG; CSP: Cotia, SP; PSP: Pirassununga, SP; TRJ: Teresópolis, RJ. *I. sca: I. scapularis; I. pac: I. pacificus; I. ric: I. ricinus.*

smaller, ranging from 678 to 686 bp for *I. scapularis* and 708 to 728 bp for *I. pacificus.* Similarly, Fukunaga et al. (2000) reported varied sequence sizes between individuals within the same or from different populations of a single *Ixodes* species.

The ITS2 sequence variations observed between the tick colonies in this study (range, 1.3–4.9%) were similar to those reported for previous studies encompassing different populations of others tick species. Wesson et al. (1993) reported that the ITS2 sequence of *I. scapularis* and *I. dammini* showed respectively 3.2 and 4.5% of intraspecific variable sites. The degree of ITS2 similarity between these two species was one piece of evidences to consider *I. dammini* a junior synonym of *I. scapularis* (Wesson et al. 1993). Among other tick genera, Barker (1998) reported nucleotide variation at 22 sites (1.9%) and 41 sites (3.5%) for the ITS2 sequences of four populations of *Boophilus microplus* (Canestrini) and six populations of *Rhipicephalus appendiculatus* (Neumann), respectively. Furthermore, other studies have shown that tick species that were thought to be conspecific in the past (due to morphological similarity) showed sequence variations substantially greater than that observed between the tick colonies analyzed in the current study. Zahler et al. (1995) reported that the ITS2 sequence variation between *Dermacentor reticulatus* (F.) and *Dermacentor marginatus* (Sulzer) was 12.2%. These two species had been considered synonymous in the past; however morphological, biological, and ITS2 sequence analysis have contributed to the validation of both species (Zahler and Gothe 1997). *Dermacentor variabilis* (Say) and *Dermacentor andersoni* Stiles, which are distinct but closely related species, showed 12.3% variation in the ITS2 sequence (Zahler et al. 1995). In fact, the three *I. ricinus*-complex species used in our analysis (as outgroups) showed a much greater sequence variation among them than that observed between our tick colonies (BMG, TRJ, CSP, and PSP). The similarity tree constructed by the neighbor-join-

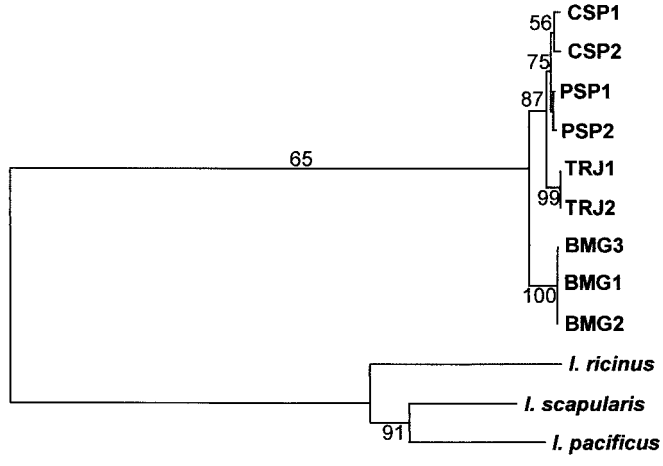

Fig. 4. Similarity tree based on ITS2 sequence data for *I. loricatus* from four localities in Brazil, compared with three different outgroup species (*I. scapularis, I. pacificus* and *I. ricinus*). The tree was constructed using the neighbor-joining method with p-distance (scale bar). The bootstrap confidence levels (from 500 replications) are shown above the branch tested.

ing method with nucleotide distances showed that our samples clustered according to geographic proximity of sites of origin and that the distances between them were similar to what would be expected for intraspecific variation found in other ticks species.

In conclusion, the morphological and biological data, in conjunction with the ITS2 analysis reported here, support the conspecificity of *I. loricatus* and *I. didelphidis.* The latter should therefore be considered a junior synonym of the former.

Acknowledgments

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