Genetic confirmation of the specific status of *Triatoma petrochii* (Hemiptera: Reduviidae: Triatominae)

Triatoma petrochii Pinto and Barreto is a Brazilian triatomine (Hemiptera; Reduviidae) that is morphologically similar to *Tri. brasiliensis* Neiva (Fig. 1). Both occur in the arid north–eastern states of Brazil, generally in rock piles associated with the large rodent *Kerodon rupestris* (Lent and Wygodzinsky, 1979). However, *Tri. brasiliensis* also invades peridomestic habitats and human dwellings, and is well-known as an important domestic vector of *Trypanosoma cruzi*, the causative agent of Chagas disease (Silveira *et al.*, 1984). In contrast, *Tri. petrochii* seems to be entirely sylvatic, without epidemiological significance.

Triatoma brasiliensis is morphologically and chromatically very variable (Lent and Wygodzinsky, 1979; Costa *et al.*, 1997), such that Lucena (1970) considered *Tri. petrochii* simply as a further variant of *Tri. brasiliensis*. This decision was challenged by Lent and Wygodzinsky (1979), who re-erected *Tri. petrochii* as a separate species because of consistent, albeit small, morphological differences from *Tri. brasiliensis*, and because cross-mating experiments between these two species failed to produce viable hybrids (Espínola, 1971). However, the validity of cross-mating experiments for determining the specific status of triatomines has been questioned (Barrett, 1996), indicating that molecular approaches might be more reliable for the determination of closely related species. It was therefore decided to compare, by allozyme electrophoresis, samples of these two putative species living in sympatry in north–eastern Brazil, to verify whether or not they are reproductively isolated.

Nine adult insects of each species were collected from rock piles (in an area of about 100 m^2) near the town of Caicó in the state of Rio Grande do Norte, Brazil, for analysis by horizontal agarose-gel electrophoresis. They were identified according to Lent and Wygodzinsky (1979) and by comparison with pinned specimens in the Herman Lent Hemiptera Collection. The head and thorax of each insect were homogenized in enzyme stabilizer for allozyme electrophoresis by the method of Shaw and Prasad (1970). After electrophoresis, the gels were stained as described by Manchenko (1994). Fourteen enzyme systems were analysed, as follows: aconitate hydratase (EC 4.2.1.3; ACON); fumarate hydratase (EC 4.2.1.2; FUM); glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6PD); glutamicoxaloacetic transaminase (EC 2.6.1.1; GOT); glycerol-3-phosphate dehydrogenase (EC 1.1.1.8; G3PD); hexokinase (EC 2.7.1.1; HK); isocitrate dehydrogenase (EC 1.1.1.42; IDH); malate dehydrogenase (EC 1.1.1.37; MDH); malic enzyme (EC 1.1.1.40; ME); mannose-phosphate isomerase (EC 5.3.1.8; MPI); glucose-phosphate isomerase (EC 5.3.1.9; PGI); phosphoglucomutase (EC 2.7.5.1; PGM); phosphogluconate dehydrogenase (EC 1.1.1.44; GDH); and xanthine oxidase (EC 1.2.3.2; XOD). Genotype frequencies were obtained directly by band counting. From these, gene frequencies, heterozygosity estimates, fits to Hardy–Weinberg equilibria and levels of genetic identity (I; Nei, 1978) were calculated by the Biosys-1 computer programme (Swofford and Selander, 1981).

Sixteen loci were observed, of which 11 were diagnostic (*sensu* Ayala, 1983) of each species (see Table and Fig. 2). Mean heterozygosities were very low (0.00 for *Tri. brasiliensis* and 0.02 for *Tri. petrochii*). The genotype proportions for the one polymorphic locus (*Acon*) showed no deviations from those expected for populations in Hardy–Weinberg equilibrium (Fisher exact test; $P > 0.05$).

Since the insects were collected from precisely the same ecotope, the large genetic differences between them indicate that

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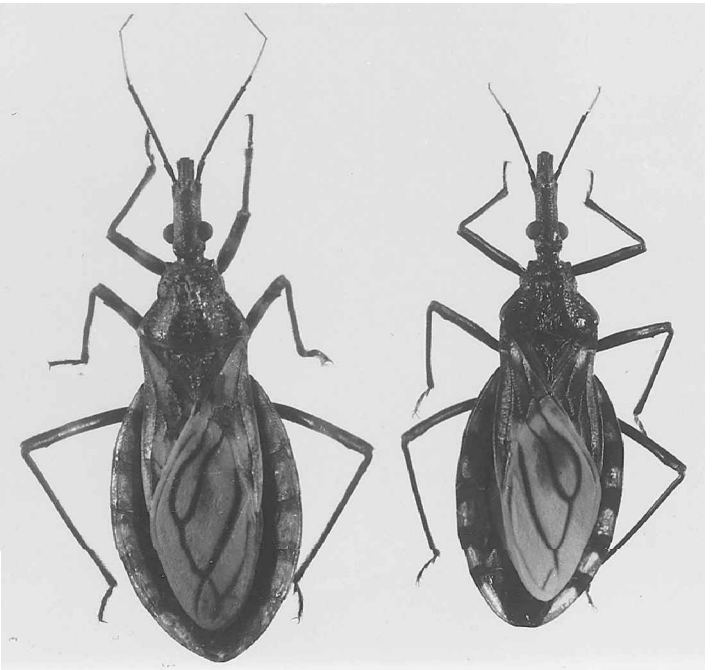

Fig. 1. Adult individuals of *Triatoma brasiliensis* (left) and *Triatoma petrochii* (right).

TABLE

Alleles found for each locus in Triatoma brasiliensis *and* Tri. petrochii

*Locus**	Triatoma brasiliensis	Triatoma petrochii
ACON1	B	A
ACON2	B	A (0.28), C (0.72)
FUM	A	B
G3PD1	A	A
G3PD2	A	A
G6PD	B	A
GOT	A	A
HK	B	A
IDH	B	A
MDH	B	A
ME	A	A
MPI	A	B
GDH	B	A
PGI	A	B
PGM	A	B
XOD	A	A

* See text for explanation of abbreviations.

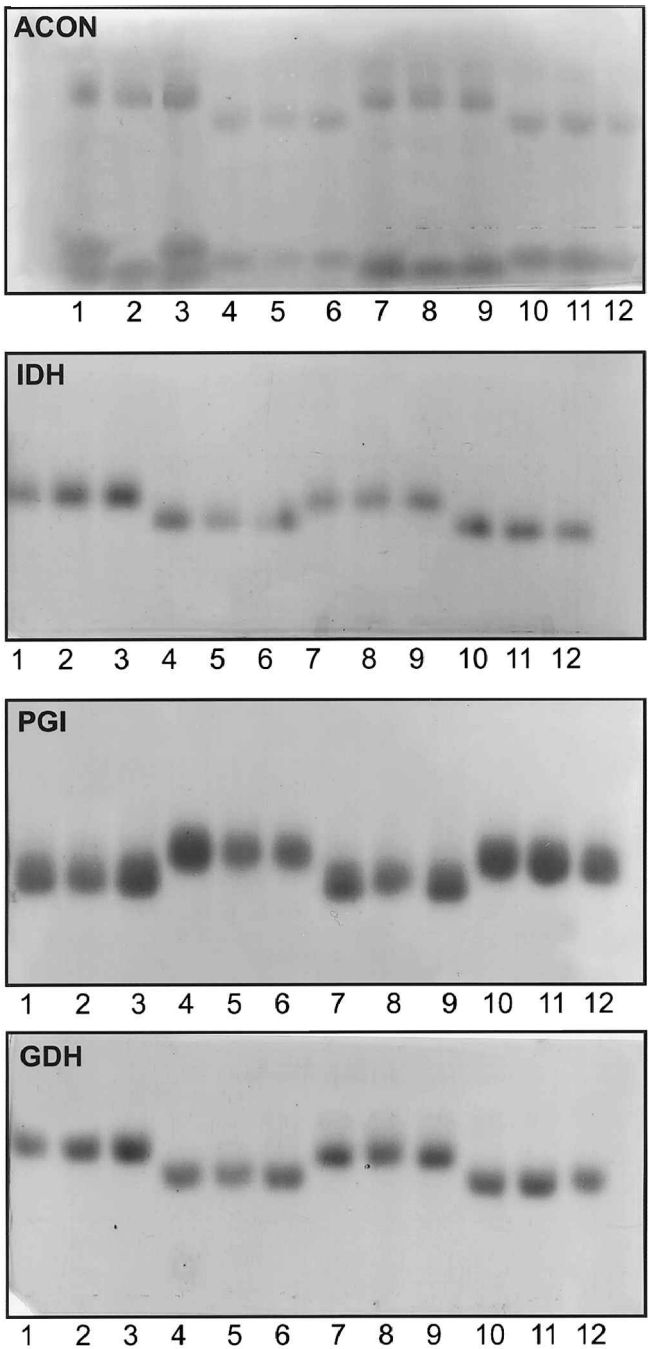

Fig. 2. Allozyme patterns for aconitate hydratases 1 and 2 (ACON), isocitrate dehydrogenase (IDH), glucose-phosphate isomerase (PGI) and phosphogluconate dehydrogenase (GDH) from *Triatoma petrochii* (lanes 1–3 and 7–9) and *Triatoma brasiliensis* (lanes 4–6 and 10–12).

Tri. petrochii and *Tri. brasiliensis* are indeed reproductively isolated, so that each should be considered a valid species. The present results are thus in agreement with the conclusions of Espínola (1971) and Lent and Wygodzinsky (1979) rather than that of Lucena (1970). Furthermore, the very low level of genetic identity observed between the two species ($I = 0.35$) indicates that, in spite of their morphological similarity, they have been evolving independently for considerable time (Thorpe and Solé-Cava, 1994).

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