Fig. 1. Localities where *An. darlingi* were collected in Brasil.

The pellet was washed with 70% ethanol, dried, and dissolved in 20 μl of distilled water. Polymerase chain reaction (PCR) for specific amplification of ITS2 was conducted according to Porter and Collins (1991) using primers CP16 (5′-GCGGGTACCATGCTTA-AATTTAGGGGGTA-3′) and CP17 (5′-GCGC-CGCGGTGTGAACTGCAGGACACATG-3′). The PCR reaction was conducted for 25 cycles (94°C for 1 min, 50°C for 2 min, and 72°C for 2 min) and the products visualized by ethidium bromide staining after electrophoresis in a 1.2% agarose gel.

The PCR products were cloned into pBluescript SK and sequenced (Sambrook et al. 1989) using the Sequenase kit 2.0 (Amersham). The sequences were aligned with the CLUSTAL W (1.6) program. To obtain a better alignment the nucleotide positions were adjusted manually. The sequence determined for mosquitoes from Sao Paulo has been deposited in the GenBank database (accession number AF032133).

Results and Discussion

Anopheles darlingi females were collected from malaria endemic regions (Boa Vista, Plácido de Castro and Porto Velho) and nonendemic regions (Dourado and Itabela) (Fig. 1). To compare their ITS2 sequences, 20 clones of PCR products obtained from *An. darlingi* genomic DNA were sequenced. Their length ranged from 461 bp (Dourado) to 476 bp (Boa Vista), and their GC contents ranged from 56.4 to 57.8%. The boundaries of ITS2 were defined, based on previously published sequences for *An. freeborni* and *An. hermsi* (Porter and Collins 1991). The *An. darlingi* ITS2 begins at position 35 and ends at position 448 of the amplified DNA (Fig. 2), being flanked by ≈34 bp of the 5.8S and 29bp of the 5′ end of 28S.

The GC content is quite conserved among mosquitoes. *An. nuneztovari* ITS2 GC content ranges from 55.3 to 55.7% (Fritz et al. 1994). Mosquitoes of the *An. quadrimaculatus* complex have their GC contents ranging between 55.1 and 57.3% (Cornel et al. 1996) and *Culex pipiens* L. and *Culex quinquefasciatus* Say ITS2 GC content is 58% (Severini et al. 1996).

No ITS2 clonal variation was observed among specimens from Dourado, Itabela, Plácido de Castro, and Porto Velho; variation for clones from Boa Vista had a value of 0.018%. Comparing this Boa Vista value to those reported by Manguin et al. (1999), this variation was low and not significant. It had 6 mismatches (5 transversions/1 transition) and 2 single-base insertion/deletions (Fig. 2).

Recently, Manguin et al. (1999) showed that, except for Belize samples, there is no evidence of sibling species when 15 populations of *An. darlingi* from South America were analyzed using isozymes, ITS2 sequences, random amplified polymorphic DNA (RAPD), and morphological markers.

In our ITS2 analysis, sequences of *An. darlingi* from Dourado were ≈10 bp shorter than the others (Fig. 2) and had a 2-bp deletion localized within the 5.8S