State of Acre, Brazil, this species predominated in a recently colonized area (Natal et al. 1992) and the malaria infection rate of *An. oswaldoi* detected by enzyme-linked immunosorbent assay was 7.8% (Branquinho et al. 1993). Recently, Marrelli et al. (1998) reported a positive correlation between the infection rate of *An. oswaldoi* with *P. vivax*-like parasites and the presence of antibodies against this variant in a human population of Acre.

Taxonomic reviews of anophelines belonging to subgenus *Nyssorhynchus* (Faran 1980, Linthicum 1988) have indicated many problems in the morphologic distinction of specimens within the Oswaldoi group. The percentage of black scaling on basal hind tarsomere II used to separate members of this group may overlap. In addition, Klein and Lima (1990) suspected that specimens identified as *An. oswaldoi* captured in open clearings in Costa Marques, State of Rondônia, Brazil, were actually *An. konderi* Galvão & Damasceno, and that *An. oswaldoi* sensu stricto was restricted to forested areas.

Anopheles konderi was regarded as a junior synonym of *An. oswaldoi;* however, recently it has been considered as a separate species of difficult morphologic distinction. Adults and immature stages of both species have almost identical morphological features, and the only reliable character for distinguishing these taxa is based on features of the apex of aedeagus (Galvão and Damasceno 1942, Causey et al. 1946).

Based on the above mentioned data, the current study was undertaken to compare the extent of differences of the ITS2 sequences of specimens of *An. oswaldoi* sensu lato from several localities in the Amazon Region, South America. The data indicate that *An. oswaldoi* is a complex of cryptic species and that DNA based identification could help to resolve the taxonomic questions related to this group.

Materials and Methods

Mosquitoes. All mosquitoes used in this study were field collected by Shannon traps or at human baits (with their informed consent), in the following localities: Linhares, Espírito Santo (Brazil) (19° 22′ S, 40° 4′ W), Plácido de Castro, Acre (Brazil) (10° 20′ S, 67° 11′ W), Costa Marques, Rondônia (Brazil) (12° 26′ S, 64° 13′ W), Novo Airão, Amazonas (Brazil) (1° 56′ S, 61° 22′ W), Santana, Amapá (Brazil) (0° 2′ N, 51° 3′ W), Ocamo (Venezuela) (3° 0′ N, 65° 70′ W), Yurimaguas (Peru) (5° 55′ S, 76° 0′ W) (Fig. 1). Species were identified according to Faran (1980) and Faran and Linthicum (1981) and immediately immersed in isopropanol. Mosquitoes were transported to the Laboratory of Insect Vectors, Department of Parasitology, University of Sao Paulo, and stored at room temperature until DNA extraction. One specimen from each locality was processed.

DNA Extraction, ITS2 Amplification, Cloning and Sequencing. Individual mosquitoes were homogenized in 50 μl of lysis buffer (100 mMTris · HCl, pH 7.5; 100 mM NaCl; 100 mM EDTA; 1% SDS; 1 mg/ml proteinase K) and incubated for 1 h at 60°C. Genomic

Fig. 1. Collection localities of the *An. oswaldoi.* (1) Linhares, Espírito Santo (Brazil); (2) Plácido de Castro, Acre (Brazil); (3) Costa Marques, Rondônia (Brazil); (4) Novo Airão, Amazonas (Brazil); (5) Santana, Amapá (Brazil); (6) Ocamo (Venezuela); (7) Yurimaguas (Peru).

DNA was extracted with phenol (2×) phenol/chloroform (1×) chloroform/isoamylic (1×), and precipitated by cold ethanol overnight. After centrifugation (5 min, 12,000 × *g*), the pellet was resuspended in 1 ml of TE containing 10 mg/ml RNase. The DNA was again precipitated by addition of 20% (vol:vol) of sodium acetate 3 M and 1 ml of isopropanol. The suspension was kept for 15 min at room temperature and centrifuged (30 min, 12,000 × *g*).

The pellet was washed with ethanol 70%, dried and dissolved in 20 μl of distilled water. Polymerase chain reaction (PCR) for specific amplification of ITS2 was conducted according Porter and Collins (1991) using the following primers: CP16 (5′-GCGGGTACCAT GCTTAAATTTAGGGGGTA-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, Cleveland, OH). The sequences were aligned with the CLUSTAL W (1.6) program (Thomson et al. 1994). Four to 5 clones from each mosquito were sequenced.

DNA Sequence Analysis. The sequence data were aligned initially using the CLUSTAL W (1.60) program. To obtain a better alignment, the obviously mis-aligned nucleotides were adjusted manually.