```
                                                  *
           GACTCAGtGcgagGTACACATTTTTGAGTGCCCACATTCACCGCAGAACCAACTAGCGAGGTCGTCGCCGCCGCCGGTCAGCCGGCgcGcgcggcT
Dourado    .......C.---A.......................................................................CG.------.  87
Itabela    ....................................................................................................  96
B.Vista    ....................................................................................................  96
P.Castro   ....................................................................................................  96
P.Velho    ....................................................................................................  96

           TAGCTGCgTACTGATGATTTGATTGACGCgcCgcGtcCCCAACCGGACGC GCCCGTGTGTGGTCAAGCATTGAAGGACTGTGGCGTGGTGGGTgC
Dourado    .......-....................--.--.CT.................................................-.  177
Itabela    ...............................................-....................................  191
B.Vista    ...............................................-....................................  191
P.Castro   ...............................................-....................................  191
P.Velho    ...............................................-....................................  191

           acCGTGTGTCGTTGCTTAATACGCGACCCTCTCTC CGGTTTCACATCTGGAGCGGGCTATCCAGTcAcAATCCCCAGCGAAATGTGCCGATACAC
Dourado    GA..............................-..............................G.G.........................  272
Itabela    ................................-...............................................................  286
B.Vista    ....................................................................................................  287
P.Castro   ................................-...............................................................  286
P.Velho    ................................-...............................................................  286

           GGGTAGCCCCGATGTGGAGaTCCAAGCGAGGACCTCCCTCAAAACCATTgTGATGAAACCCCACCACACAAGAGAGAAGAGAGAGAGCGACCAAAA
Dourado    ...............................................A....................................  368
Itabela    ....................................................................................................  382
B.Vista    ..................T.................................................................................  383
P.Castro   ....................................................................................................  382
P.Velho    ....................................................................................................  382

                                                                                  *
           GCAACGTTCGCACGCGCTGTCAGCTCATCGAGcGcGCACACGGAtCTAGGAACTAGGATCTCAAGTGGGCCTCAAATAATGTGTGACTACCCCC
Dourado    ...............................G....................................................  461
Itabela    ...........................................C.......................................  475
B.Vista    ....................................................................................  476
P.Castro   ....................................................................................  475
P.Velho    ................................-...................................................  474
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Fig. 2. Alignment of nucleotide sequences (5′ to 3′) of the ITS2 and flanking regions of *An. darlingi* from several states. Asterisks indicate the limits of ITS2.

region. Substantial differences were observed in the ITS2 sequence of anophelines captured in Dourado when compared ITS2 from mosquitoes captured in the other 4 localities. These differences ranged from 4.64% (Dourado-Plácido de Castro) to 5.48% (Dourado-Itabela) primarily in the ITS2 region.

Sibajev-Freitas et al. (1995) compared the cycle of biting activity of geographically distinct *An. darlingi* populations and concluded that they were different. In Dourado, where malaria is not endemic, females displayed an exophilic behavior and trimodal biting cycle, whereas females from malarial areas such as Costa Marques were captured indoors, exhibiting a bimodal biting cycle (Rosa-Freitas et al. 1992). Our findings indicated that all the *An. darlingi* captured in the Amazon region (Boa Vista, Plácido de Castro and Porto Velho) and in the northeast of Brazil (Itabela) had almost identical ITS2 DNA sequences. However, we observed a high pairwise sequence difference in the ITS2 of mosquitoes from Dourado, compared with the ITS2 of other populations analyzed in this study.

The results on the ITS2 sequences confirmed the previously described differences of the Dourado *An. darlingi* population, when compared with other Brazilian populations (Sibajev-Freitas et al. 1995). Further analysis is needed to determine whether the Dourado population is a morphologically similar sibling species related to one found in the North and Northeast of Brazil.

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