

Tephritid fruit flies of the genus *Ceratitis* MacLeay (Dacinae: Ceratitidini) pose one of the greatest threats to Africa's horticultural industry. To curb their economic injury and prevent spread to new areas, it is vital that quarantine workers and developers of control tools and strategies can unambiguously identify the different species at any life stage. However, the classification of Tephritidae to the species level based on morphology alone continues to be difficult. We have sought to utilize molecular markers, which are life-stage independent, stable and intrinsic to the organisms, for this purpose. Consequently, we screened 24 Medfly (*Ceratitis capitata*) microsatellite markers for cross-species amplification in *C. rosa*, *C. fasciventris* and *C. cosyra*. The presence or absence of each locus in each species was tested by PCR, and negative results confirmed by Southern analysis. Locus homology with the corresponding Medfly loci was confirmed by sequencing. Our findings indicate that it is possible to distinguish the different species examined using a combination of the Medfly-based primers. Of the 24 loci screened, 17 were common to all, five were absent in *C. cosyra*, three were absent in *C. rosa*, two were absent in *C. fasciventris*, while one locus was present only in *C. capitata*. In addition, sequence results indicate that most of the Medfly-based microsatellite markers could be useful for population genetic studies in the species tested, an aspect which would facilitate the tracing of the geographical origin of colonist pest populations, assessment of their invasive potential and risk assessment.