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# Use and trends of molecular markers in sandflies (Diptera: Psychodidae)

## *Usos y tendencias de marcadores moleculares en flebotomíneos (Diptera: Psychodidae)*

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*RESUMEN*

La subfamilia Phlebotominae está compuesta principalmente por los géneros *Lutzomyia* y *Phlebotomus*, vectores principales de patógenos virales, bacterianos y protozoarios. Desde los años 90 marcadores moleculares han ayudado a abordar problemas dentro del taxón, como por ejemplo: determinar la estructura genética, resolver conflictos sistemáticos, especiación, co-evolución de parasito y vector. Esta revisión pretende crear un compendio de la investigación realizada con marcadores moleculares en este grupo taxonómico, para así facilitar el trabajo de investigadores que pretenda identificar marcadores y el análisis de datos apropiados para responder sus preguntas. La tendencia principal encontrada fue el uso de ADN mitocondrial como marcador molecular, la secuenciación de ADN como técnica de caracterización y el análisis filogenético como método de análisis predilecto. La mayoría de los estudios revisados se centran en la especie *Lutzomyia longipalpis*, vector principal de leishmaniosis visceral en las regiones tropicales de América, y *Phlebotomus papatasi* vector principal de leishmaniosis cutánea en Europa, Asia y América. Los problemas taxonómicos y las descripciones de estructura genética fueron los problemas más abordados por los investigadores, seguidos por la resolución de conflictos sistemáticos. La investigación a futuro empleando marcadores moleculares en flebotomíneos deben apuntar hacia: el desarrollo de barcoding genético como técnica complementaria a la identificación morfológica y la secuenciación de genomas para así avanzar en el área de relaciones parasito-vector.

**Palabras clave:** Marcadores moleculares, flebotomíneos, leishmaniosis, *Lutzomyia*, *Phlebotomus*.

SUMMARY

*The subfamily Phlebotominae is principally composed of the Lutzomyia and Phlebotomus genera: the main vectors of several protozoan, bacterial and viral pathogens. Since the 1990's molecular markers have enabled us to effectively address many issues concerning this taxon by, for example, solving systematic conflicts, increasing our understanding of speciation and host-parasite co-evolution, and determining the genetic structure of populations. In this paper we review the research undertaken using molecular markers in this taxonomic group. We hope that this will make it easier for scientists to identify markers and data analyses appropriate to their particular research interests. The principal trends we found are a move towards the use of mitochondrial DNA as molecular markers, DNA sequencing as the characterization method of choice, and phylogenetic analysis for analyzing the data. Most of the studies reviewed center on Lutzomyia* longipalpis*, the main vector for visceral leishmaniasis in the American tropics and Phlebotomus papatasi, the main vector for cutaneous leishmaniasis in Europe, Asia and Africa. Taxonomic problems and the description of genetic structure are the issues most addressed by researchers, followed by resolving systematic conflicts. Future research using molecular markers in the study of sandflies should be aimed towards: a) the development of genetic barcoding as a complementary tool for morphological identification and b) genome sequencing to increase our understanding of host-parasite interactions.*

***Key words:*** *Molecular markers, sandflies, leishmaniasis, Lutzomyia, Phlebotomus.*

## INTRODUCTION

Sandflies of the Phlebotominae sub-family Rondani 1840 are vectors of leishmaniasis, bacterial and viral diseases. The genres *Phlebotomus* Loew 1845 and *Lutzomyia* França 1924 are mainly involved in the transmission of several species of the *Leishamania genus* (Young & Duncan, 1994). The distribution of the genus *Phlebotomus* is mainly restricted to the old world (Europe, African and Asia continents) and contains few species, in contrast with the genus *Lutzomyia* which is restricted to the Americas and

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contains around 400 described species (Ready, 2000; Young & Duncan, 1994). Mostly all taxonomic descriptions of sandflies are based on morphologic characters. The genus *Lutzomyia* contains a lot of species complex and groups of cryptic species, a few with lower variability and phenotypical plasticity, blurring the systematic organization and taxonomic separation (Bauzer *et al.*, 2002; Maingon *et al.*, 2007; Uribe, 1999; Yin *et al.*, 2000). The taxonomy of the group has a great epidemiological importance since not all species are proven vectors, and the ones that have been proven so far possess differences in bite behavior or host preference (Rabinovich & Feliciangeli, 2004). Therefore, the clarification of the taxonomic and systematic classification of the group is the main purpose of mostly all studies involving sandflies.

Over the past 30 years, the study of sandflies has been mainly enriched using molecular markers, mostly isozymes and DNA markers (using techniques ranging from RAPD-PCR since 1994 to sequences analysis nowadays), allowing researchers to overcome the limitations of morphology and classic ecology studies, opening new insights specially on taxonomy and systematic of the group (Adamson *et al.*, 1991; Arrivillaga *et al.*, 1995; Mahamat *et al.*, 1992; R. Ward & Miles, 1978). At the present time, the great importance that molecular markers have in the study of sandflies is clear. More than 100 articles have been published using molecular markers allowing for the collection of more than 4,500 DNA sequences stored in GenBank (Benson *et al.*, 2005), providing thousands of characteristics to relate or separate populations, species, groups or genera; this is obviously more than morphologic characteristics can provide.

However, the use of molecular markers doesn't assure a robust systematic classification or definition of the taxonomic status, i.e. some genes used in systematic studies have been demonstrated to be better than others for reconstructing phylogenies among insect taxa (Simon *et al.*, 1994), while other genes have been proven useless on a taxonomic level even though they have been used in several articles (Golczer & Arrivillaga, 2010). Consequently, a careful evaluation of the molecular marker used or the analysis technique applied is needed at the beginning and end of a study, especially on sandflies, due to the small amount of tissue available to isolate DNA or enzymes to reproduce or correct findings (Golczer & Arrivillaga, 2008).

This article is part a review and part an original synthesis of more than 89 original articles (Table I), first reviewing the use and classification of molecular markers using sandflies articles as examples, then discussing their use and the analysis of the data employed and analyzing the limitations of use, and finally presenting a compilation of PCR primers used in the articles (Table II). The first part is written to allow those familiar with molecular markers to skip it and read the subsequent discussion. Researchers studying sandflies using molecular markers will find this marker compilation useful to facilitate the selection process and assess which markers have been developed, and which of those meet the requirement to answer the question or test the hypothesis stablished.

## MOLECULAR MARKERS

A molecular marker is a molecular characteristic of an organism that can be viewed or measured, directly or indirectly using a technique, that provides genotypic information that enable researchers to monitor, differentiate, classify and establish genealogical or phylogenetic relationships; these markers can be classified into biochemical or genetic (molecular) categories and their use is dependent of the question that researchers want to address with them (Avise, 1994; Parker *et al.*, 1998; Walker & Rapley, 2000) .

The most-used biochemical markers are isozymes. These enzymes vary in structural patterns but not on function per se. They are isolated from individuals in an electrophoresis gel and used as Mendelian characteristics or identity patterns, allowing researchers to make inference based on allele frequency, genetic flow and population genetics (Arrivillaga *et al.*, 2003; Avise, 1994; Dujardin *et al.*, 1999; Hillis *et al.*, 1996; Mazzoni *et al.*, 2002).

Genetic markers are more versatile than isozymes because they are evaluated by a variety of techniques such as RFLP (Restriction Fragment Length Polymorphism) (Aransay *et al.*, 1999), DNA Strand Hybridization (Maingon *et al.*, 1993), RAPD-PCR (Random Amplification of Polymorphic DNA) (Adamson *et al.*, 1993; Dvorak *et al.*, 2006; Maingon *et al.*, 1993), SSCP (Single Strand Conformation Polymorphism) (Arrivillaga *et al.*, 2003), Microsatellites (Aransay *et al.*, 2003; Day & Ready, 1999; Hamarsheh *et al.*, 2006; Maingon *et al.*,

2003; Watts *et al.*, 2005) or DNA Sequencing (Vivero *et al.*, 2007). The genetic markers can be classified according to the location of the genome analyzed, such as the mitochondrial genome or nuclear genome. The difference of inheritance patterns can be very useful in taxonomic, systematic, phylogenetic or phylogeographic studies (Avise *et al.*, 1987; Beati *et al.*, 2004; Esseghir *et al.*, 1997; Kambhampati & Smith, 1995; Simon *et al.*, 1994; Togerson *et al.*, 2003).

## DISCUSSION OF MOLECULAR MARKERS USE IN SANDFLY RESEARCH

It's clear that molecular markers (DNA & RNA) have been mainly used by researchers investigating questions about sandflies: 76 of 89 (85%) articles reviewed in this work show this tendency towards the use of DNA and RNA, with isozyme or protein sequences accounting for the rest (13 articles reviewed, 14%). Regarding the use of mitochondrial DNA, only 31% (28 articles) of the articles reviewed use exclusively mitochondrial sequences as markers. The rest (48 of 89) employ a at least a form of nuclear marker such as: nuclear DNA sequences, RAPD, RFLP, SSCP and microsatellites.

There has been a recent increase in the number of articles describing the use nuclear sequences in sandflies, especially in the per and cac regions (Bauzer *et al.*, 2002; Bauzer *et al.*, 2002; Lins *et al.*, 2002; Mazzoni *et al.*, 2002; Mazzoni *et al.*, 2006). These markers, called "clock genes", are involved in the circadian rhythm and temporal regulation of processes, specifically the mating process (Ritchie *et al.*, 1999). These genes have been proven non-informative in phylogenies at a taxonomic level on the *L. longipalpis* complex, showing no divergence and thus showing no evidence of fixation which will be the signature for an isolation process between taxa (even at a geographic scale) (Golczer & Arrivillaga, 2010). Although these clock genes have also been used on other species such as *Lutzomyia intermedia* and *Lutzomyia whitmani*, the authors highlight the low bootstrap values on the nodes of the phylogenetic reconstruction (Mazzoni *et al.*, 2006), and it is important to remark that the method use was Minimum Evolution were the data is transformed into pairwise distance which has been proven to be less robust than other methods such as Maximum Likelihood (Yang, 2006). Other nuclear genes such as 28Sr, 18Sr and ITS are most often used at the level of species, groups or genera, mostly in articles describing research with a systematic focus due to the low mutation rate (Caterino, *et al.*, 2000) .

The most common technique used on phylogenetic studies is DNA sequencing. In comparison with isozymes and RNA markers, this technique has more precision for detecting variability, similarity and phylogeny between species or groups (Caterino *et al.*, 2000); in contrast with isozymes which have a high mutational rate (Hillis *et al.*, 1996).

The questions addressed by researchers have been in relation to genetic structures and species identification (30 of 89 articles reviewed, 44%), beta taxonomy and systematics (13 of 89 articles, 15%) and phylogenetic reconstruction and species delimitation (46 of 89 articles, 51%). The preference for molecular marker used has a tight link between the problems addressed and the limitations of such markers. The questions regarding genetic structures can be explored using various kinds of markers (DNA sequence, isozymes, etc.) and the choice made by the researchers tends to depend on the experience and the light that the marker shed on similar questions in other systems. However, the systematic inquiries are restricted to DNA sequences of low mutation rate, avoiding the risk of saturations and homoplasy (McDowall, 1973).

The species studied most in-depth within the sandflies has been *L. longipalpis* (32% of articles reviewed). This trend is based in its epidemiological importance, its widespread presence (from Central America to Argentina) and its conflictive taxonomy. Some research declares it as a species complex (Arrivillaga *et al.*, 2000) whereas others differentiate some populations declaring them as different species (Arrivillaga & Feliciangeli, 2001).

Those facts reveal two main goals of sandfly researchers: The need to link their results to epidemiological records and applications, and the need to clarify the taxonomy and systematic classification of the group. The use of molecular markers to develop epidemiological applications arises from the hypothesis that links the vectorial capacity to transmit some *Leishmania* species and the genetic structure of the sandfly populations (Arrivillaga *et al.*, 2003; Ishikawa *et al.*, 1999). The need for resolution of the taxonomic and systematic issues within the group becomes such an important issue due to the great amount of species within the *Lutzomyia genus*, or the

great similarity between the morphology of the genus that belongs to the Phlebotominae sub-family, which doesn't allow for a clear identification of the supra-specific and sub-specific groups (Bejarano, 2001; Depaquit *et al.*, 1998; Depaquit *et al.*, 2000).

The countries in which research of sandflies using molecular markers is held reveals a clear tendency in which UK and Brazil are leading the field with 40% and 30% of publications, respectively. Only 12% of articles are produced solely in developing countries. This latter fact reveals the great cost needed to invest on structure and reagents used in molecular techniques, accounting for why 73% of the articles reviewed are produced in collaboration between European and/or North American authors with Latin American authors.

## DISCUSSION OF DATA ANALYSIS TECHNIQUES

The use of a molecular marker alone (in any field) doesn't assure the success of a research project. The posterior analysis of the data provided by those markers reveals the results, giving the same importance to the choice of a molecular marker as to the choice of an analytical tool. Based on this review, phylogenetic analysis was predominant over population genetics analysis.

Isozyme markers provide a kind of information that could be used in population genetics, distance and phylogenetic analysis. But with the latter, the kind of data produced requires special treatments to compare DNA sequences (Nei & Kumar, 2000). In consequence, the analysis of this kind of data in the articles reviewed is biased to distance analysis rather than in phylogenetic analysis.

Distance analysis (UPGMA and Neighbor Joining methods) can be employed with data from RAPD and microsatellites using the pattern shown in the electrophoresis gel as characters (Khuner & Felsenstein, 1994). This kind of data was also used by de Azevedo *et al.* (2000) and Cárdenas *et al.* (2001). A striking aspect of the articles reviewed is the preference for distance analysis and it's definition as a phylogenetic method as Mazzoni *et al.* (2006), Bauzer *et al.* (2002; 2002), Lins *et al.* (2002), and Parvizi & Assmar (2007), Seblova *et al.* (2012), Sacarpassa & Alengcar (2013), Nzelu *et al.* (2015) among others. The misinterpretation of the data reflects the doubt about the phylogenetic principles used to analyze the data, leading to conclusions not based in any phylogenetic principle (Depaquit *et al.*, 1998).

Also, it is observed that there is a recurring error employing the bootstrap technique (used for statistical support). Aransay *et al.* (2000) use 100 bootstrap replicates, when a minimum of 500 replicates is established as statistically robust (Soltis & Soltis, 2003). When applying phylogenetic or distance analysis, these articles sometimes don't report the parameters used in their computer analysis (Bauzer *et al.*, 2002; Bauzer *et al.*, 2002) or the statistical support on the nodes or branch of the trees, affecting the ability to replicate and verify the analysis. This issue affects the reliability of the article at the same degree as if the analysis was performed with 100 bootstrap replicates. The aforementioned issues regarding the analytical tools employed by various authors reside in the superficial knowledge of their parameters and assumptions (Yang, 2006). Authors should explore the seminal articles and basic literature of the techniques they wish to employ, resulting in a better understanding of the input options given to the software that will perform the calculations instead of just repeating the same steps of the latest article in the field.

## LIMITATIONS EMPLOYING MOLECULAR MARKERS IN SANDFLIES

The main restrictions that sandflies pose as a system for molecular studies is the size of the tissue/body, as it is so little that it is very difficult to isolate enough DNA to amplify microsatellite regions, nuclear regions with specific primers or RAPD (Golczer & Arrivillaga, 2008). Other restrictions, such as preservation of tissue, arise in molecular studies with sandflies (Depaquit *et al.*, 2000). Only 8 articles make note of the preservation method: storage with isopropanol (Surendran *et al.*, 2005); storage with ethanol between concentrations of 70% and 100% (Beati *et al.*, 2004; Testa *et al.*, 2002; Watts *et al.*, 2005); and dry storage in a liquid nitrogen tank (Cárdenas *et al.*, 2001; G. Lanzaro *et al.*, 1998; Meneses *et al.*, 2005). This preference is important if the studies want to use isoenzymes as a marker, given that a sandfly preserved in ethanol can't be used for studies involving isoenzymes (Testa *et al.*, 2002). The small size of sandflies affects the quantity of markers used in a given individual, as an example the average weight of a sandfly is 70 µg, and using Golczer

and Arrivillaga (2008) protocol 373 ng of DNA in average can be isolated, since 20 to 100 ng of DNA necessary to run a PCR reaction (depending of the quality, fragmentation of DNA and number of copies of the loci) only a few markers can be amplified using PCR techniques. This affects the ability to combine different markers with different resolution to support the conclusion of an article, helping to explain why only 10% of the articles reviewed employ two kinds of markers (nuclear and mitochondrial sequences).

## FUTURE PERSPECTIVES

As the use of molecular markers keeps growing, the molecular tool's costs decrease, and computational tools increase in complexity and power, researchers should use more than one molecular marker to address one question, thus increasing the robustness of the result. The use of a lot of molecular markers in a study isn't good enough by itself; it must be accompanied by a proper analysis that is fitting to the nature of the marker, the question addressed and the sample size.

The developing process of new primers could be improved by using new techniques like double digest RADseq (Peterson *et al.*, 2012), which has been proven useful to develop markers for population genetics or genetic mapping without a reference genome. Another sequencing technique that might improve our understanding of genetic variation within a species or genus of sand flies is parallel sequencing of pooled DNA samples (Futschik & Schlötterer, 2010), which overcomes the limitation of tissue size of these.

## FINAL REMARKS AND SUGGESTIONS

In our opinion, researchers that want to provide DNA barcoding tools for identification purposes should first validate the taxonomic status of the species, and as a subsequent step evaluate different genetic regions as barcodes. Without proof of the validity or cohesiveness of the taxonomic group in question, the process of testing the potential barcodes could be obscured by the presence of a species complex or groups of different morphological species which lack genetic validity (e.g. product of an incomplete speciation process) (Meyer and Paulay, 2005).

To test the validity of a species the use of more than one molecular marker is necessary. At least one mitochondrial and one nuclear marker as these two types could reveal different evolutionary events (Moore, 1995). An example of the latter is Testa *et al.* (2002); although the goal wasn't barcoding per se, the use of two types of markers enriched the conclusions at which the authors arrived. On the other hand, if the hypothesis to test is phylogeographic, mitochondrial markers should be used without the addition of nuclear markers due to the maternal inheritance. An example of the latter case is shown in the research of Arrivillaga *et al.* (2002).

While the COI (cytochrome oxidase I) region has been widely proven in insects as an useful genetic barcode (Pentinsaari *et al.*, 2014, Porter *et al.*, 2014), where it has been employed in various Phlebotomine species (17 articles of 89 reviewed), its accuracy decreases if the species included haven't been previously validated by genetic markers. As an example, Contreras-Gutiérrez *et al.* (2014) couldn't differentiate between *L. youngi* and *L. spinicrassa*, since their taxonomic status is still not clear (Golczer, 2011).

In order to perform systematic studies, different markers with different rates of evolution resolve relationships at different levels. Faster evolving sequences (such as NAD, EF, COI and cytb) work better at resolving low level relationships (species, sister taxa and some sub-generic relationships). More conserved regions (18S, 28S, among others) perform best at resolving higher level relationships (at family and genus level) (Nei and Kumar, 2000). An excellent article to reference *Lutzomyia* systematic research is Beati *et al.* (2004), not only because of the variety of markers employed, but also for the appropriate analysis of the data using different phylogenetic methods, adding more robustness to the result by showing congruence between different methods.

Lastly, in order to asses if phlebotomine species are still in the process of speciation or, if the have already completed this process, the phylogenetic analysis (using markers with different rates) should be accompanied by genetic structure analysis based on markers with Mendelian characteristics (SNPs, Microsatellites, Isozymes). These can provide robust evidence of gene flow and migration (or the lack of) between populations of different species. Naturally, the size limitation mentioned above confines the development of such markers, but this

can be counteracted by next generation sequencing techniques, which provide the necessary tools for developing and increasing sample size of future studies.

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REFERENCE TABLE

Below we offer a synthesis table showing the molecular marker used (since 1990) and its category, the kind of problem/question addressed, how many species or groups were studied, and the article of reference, hoping that it will be an important tool for future research employing molecular markers on sandflies.

**Table I. Synthesis table showing the molecular marker used species or group studied and problem/ question addressed.**

| Authors | Year | Species or Group Studied | Type of Marker | Problem/ Question Addressed | Marker(s) |
|---|---|---|---|---|---|
| Kreutzer *et al.* | 1990 | *Verrucarum* Species Group | Isozymes | Genetic Structure | 21 loci |
| Adamson *et al.* | 1993 | *L. youngi* | RAPD | Taxonomic Identification & Barcoding | N/A |
| Maingon *et al.* | 1993 | *L. youngi, L. spinicrassa* & *L. townsendi* | RAPD | Taxonomic Identification & Barcoding | N/A |
| Essseghir *et al.* | 1997 | *Phlebotomus* | mitochondrial DNA sequences | Genetic Structure | cyt b & NADH1 |
| Depaquit *et al.* | 1998 | 3 species of *Lutzomyia*, 5 species of *Phlebotomus* & 1 species of *Sergentomyia*. | mitochondrial DNA sequences | Systematics | D2 nuclear partial region of 28s) |
| Lanzaro *et al.* | 1998 | *L. longipalpis* | Isozymes | Genetic Structure | Aconitase-2 Fumarase a-Glycerophosphate dehydrogenase Glutamate oxaloacetate transaminase-1 Glutamate oxaloacetate transaminase-2 Phosphoglucoismerase Glyceraldehyde-3-phosphate dehydrogenase Hexokinase Isocitrate dehydrogenase-1 Isocitrate dehydrogenase-2 Malic acid dehydrogenase-1 Malic acid dehydrogenase-2 Malic enzyme-1 Phosphoglucomutase Trehalase |
| Mukhopadhyay *et al.* | 1998 | *L. longipalpis* | Isozymes | Genetic Structure | Aat-2, Gpi, Idh-2, Me, Aat-2 Ak Ark Gpd Gpi Hk Idh-2 Me Mdh-2, Mpi, Pgm |
| Dias *et al.* | 1998 | *L. longipalpis* | RAPD | Genetic Structure | Operon P |
| Aransay *et al.* | 1999 | 7 species de *Phlebotomus* | RFLP | Systematics | N/A |
| Lampo *et al.* | 1999 | *L. longipalpis* | Isozymes | Speciation & Divergence | Ak Ark Gpd Gpi Hk Idh-2 Mdh Me |
| Day & Ready | 1999 | *L. whitmani* | Microsatellites | Genetic Structure | AAT-class |

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| | | | | | |
|---|---|---|---|---|---|
| Ishikawa *et al.* | 1999 | *L. whitmani* | mitochondrial DNA sequences | Genetic Structure | cyt & NADH1 |
| Dujardim *et al.* | 1999 | *Lutzomyia*, *Phlebotomus*, *Sergentomyia*, *Brumptomyia* & *Warileya* | Isozymes | Systematics | ALDH, AP, HK, GPD,GPI, IDH, ME, MDH, PEP, PGM, XDH & XO |
| Mutebi *et al.* | 1999 | *L. longipalpis* | Isozymes | Genetic Structure | 16 loci |
| Depaquit *et al.* | 2000 | 11 *Phlebotomus* species | nuclear DNA sequences | Systematics | ITS 2 |
| Yin *et al.* | 2000 | *L. longipalpis* | RNA sequences | Speciation & Divergence | ARNm del peptido salivar maxadilian |
| de Azevedo *et al.* | 2000 | *L. longipalpis* | Isozymes | Genetic Structure | malic enzyme (ME), phosphogluconate dehydrogenase (GPD), glucose-6-phosphato isomerase (GPI), phosphoglucomutase (PGM), glicerol 3-phosphate isomerase (μ-GPD), hexokinase (HK), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH) & mannose 6-phosphate isomerase (MPI) |
| Di Muccio *et al.* | 2000 | *Phlebotomus perniciosus*, *P. ariasi* & *P. perfiliewi perfiliewi* | nuclear DNA sequences | Systematics | ITS2 |
| Aransay *et al.* | 2000 | *Phlebotomus* & *Lutzomyia* | nuclear DNA sequences | Systematics | 18S |
| Arrivillaga *et al.* | 2000 | *L. longipalpis* | Isozymes | Genetic Structure | adenylate kinase, arginine kinase , glucosephosphate isomerase, hexokinase , isocitrate dehydrogenase, malate dehydrogenase, & malic enzyme |
| Essseghir & Ready | 2000 | subgenus *Larroussius* | mitochondrial DNA | Speciation | COI, ITS-Rdna |
| Uribe *et al.* | 2001 | *L. longipalpis* | mitochondrial DNA sequences | Speciation | ND4 |
| Oliviera *et al.* | 2001 | *L. longipalpis* | nuclear DNA sequences | Speciation & Divergence | cac |
| Cárdenas *et al.* | 2001 | *L. shannoni* | Isozymes | Genetic Structure | Glycerol-3-phosphate dehydrogenase Malate dehydrogenase Malic enzyme Isocitrate dehydrogenase Aspartate aminotransferase Hexokinase Arginine kinase Adenylate kinase Esterase Fumarate hydratase Glucose phosphate isomerase |

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|---|---|---|---|---|---|
| Bauzer *et al.* | 2002 a | *L. longipalpis* | nuclear DNA sequences | Speciation & Divergence | per |
| Bauzer *et al.* | 2002 b | *L. longipalpis* | nuclear DNA sequences | Speciation & Divergence | per |
| Lins *et al.* | 2002 | 8 species of *Lutzomyia* | nuclear DNA sequences | Systematics | cac |
| Mazzoni *et al.* | 2002 | 9 species of *Lutzomyia* | nuclear DNA sequences | Systematics | per |
| Arrivillaga *et al.* | 2002 | *L. longipalpis* | mitochondrial DNA sequences | Phylogeny | cyt Oxidase I (COI) |
| Depaquit *et al.* | 2002 | *P. sergenti* & *P. similis* | nuclear DNA sequences | Genetic Structure & Divergence | ITS 2 |
| Mutebi *et al.* | 2002 | *L. longipalpis* | Isozymes | Genetic Structure | Gpi, Aat-1, and Pgm, |
| Testa *et al.* | 2002 | *L. youngi*, *L. townsendi*, *L. columbiana*. *L. evansi* & *L. ovallesi* | mitochondrial DNA sequences & ADNg | Taxonomic Identification & Barcoding | cyt b & EF Alfa |
| Togerson *et al.* | 2003 | 8 species of L. & 2 of *Brumptomyia* | DNA sequence & isozymes | Systematics | cyt b & Alozymes: adenylate kinase (Ak, E.C.2.7.4.3), arginine kinase (Ark, E.C.2.7.3.3), isocitrate dehydrogenase (Idh,E.C. 1.1.1.42), glycerol-3-phosphate dehydrogenase (Gpd,E.C. 1.1.1.8), malate dehydrogenase (Mdh, E.C. 1.1.1.37) & phosphoglucomutase (Pgm, E.C.5.4.2.2) |
| Hodgkinson *et al.* | 2003 | *L. longipalpis* | mitochondrial DNA sequences | Molecular Characterization | cyt b |
| Arrivillaga *et al.* | 2003 | *L. longipalpis* | mitochondrial DNA sequences, SSCP e Isozymes | Taxonomic Identification & Barcoding | cyt Oxidase I, 12S, 16S & Isozymes: hexokinase, isocitrate dehydrogenase- one, malic acid dehydrogenase-1, malic acid dehydrogenase-two, malic enzyme-1, phosphoglucoisomerase & alpha-trehalase |
| Maingon *et al.* | 2003 | *L. longipalpis* | Microsatellites | Genetic Structure | LIST6002, LIST6004, LIST6006 & LIST6012 |
| Parvizi *et al.* | 2003 | *P. papatasi* | mitochondrial DNA sequences | Genetic Structure | cyt b , NADH & wsp gene (Wolbachia) |
| Aransay *et al.* | 2003 | *P. perniciosus* | Microsatellites, Isozymes & ADNmit | Genetic Structure | AAm13, AAm20, AAm82 & AAm24 |
| Beati *et al.* | 2004 | 17 species of *Lutzomyia* | mitochondrial DNA sequences & ADNg | Systematics | 12Sr & 28Sr |

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|---|---|---|---|---|---|
| Belen *et al.* | 2004 | *P. papatasi* | Alozymes | Genetic Structure | MDH (EC 1.1.1.37), ME (EC 1.1.1.40), PGM (EC 5.4.2.2 ), EST (EC 3.1.1) & HK |
| Bottecchia *et al.* | 2004 | *L. longipalpis* | nuclear DNA sequences | Speciation & Divergence | cac |
| Yahia *et al.* | 2004 | *P. sergenti* | mitochondrial DNA sequences | Genetic Structure | cyt b |
| Meneses *et al.* | 2005 | *L. intermedia* | mitochondrial DNA sequences, SSCP & RAPD | Genetic Structure | COI, 12S , 16S & 6 RAPD primers |
| Watts *et al.* | 2005 | *L. longipalpis*, *L. pseudolongipalpis* & *L. cruzi* | Microsatellites | Genetic Structure | N/A |
| Surendran *et al.* | 2005 | *Phlebotomus* argentipes | nuclear DNA sequences | Molecular Characterization | 18S ADNr |
| Depaquit *et al.* | 2005 | *Phlebotomus canaaniticus P. economidesi* & *P. mascittii* | mitochondrial DNA sequences | Systematics | ND4 |
| Mazzoni *et al.* | 2006 | *L. intermedia* & *L. whitmani* | nuclear DNA sequences | Introgretion | per |
| de Queiroz Balbino t col | 2006 | *L. longipalpis* | RAPD | Genetic Structure | Operon P (serie de cebadores) |
| Hamarsheh *et al.* | 2006 | *Phlebotomus papatasi* | Microsatellites | Genetic Structure | M13 |
| Parvizi & Ready | 2006 | *Phlebotomus papatasi* | mitochondrial DNA sequences | Genetic Structure | cyt b, ARNt ser |
| Dvorak *et al.* | 2006 | *Phlebotomus sergenti* | RAPD | Genetic Structure | 81F & 691R |
| Vivero *et al.* | 2007 | 7 species *Lutzomyia* | RNA sequences | Systematics | ARNt ser (mitochondrial) |
| Parvizi & Assmar | 2007 | 8 species of *Phlebotomus* | nuclear DNA sequences | Taxonomic Identification & Barcoding | EF Alfa |
| Ramalho-Ortigao *et al.* | 2007 | *L. longipalpis* | cDNA & protein sequences | Molecular Characterization | V-TPAse |
| Hamarsheh *et al.* | 2007 | *P. papatasi* | mitochondrial DNA sequences | Genetic Structure | cyt b & NADH1 |
| Moin-Vaziri *et al.* | 2007 | *P. sergenti* | mitochondrial DNA sequences | Genetic Structure | cyt b, ARNt ser & NADH1 |
| De Souza *et al.* | 2007 | *L. intermedia* | RAPD | Genetic Structure | N/A |
| Mazzoni *et al.* | 2008 | *L. intermedia* & *L. whitmani* | nuclear DNA sequences | Introgretion | Ca1D, per, cac, Rp49, RpL17A, RpL36, RpS19a, TfIIA -L, up, zetacop |
| Coutinho-Abreu *et al.* | 2008 | *L. longipalpis* | mitochondrial DNA sequences | Speciation | cyt b |
| Depaquit *et al.* | 2008 | *Phlebotomus* | mitochondrial DNA sequences | Genetic Structure | ITS 2 & ND4 |
| Lins *et al.* | 2008 | *L. longipalpis* | nuclear DNA sequences | Speciation & Divergence | par |

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|---|---|---|---|---|---|
| Hernandez *et al.* | 2008 | *L.* (*verrucarum* group) | Isozymes | Genetic Structure | Mdh, Me, Idh, 6-pgdh, Aat, Hk, Ark, Ak, Pgm, Est, Fum, Aco, Gpi |
| Parvizi & Amirkhani | 2008 | *Sergentomyia* sintoni | mitochondrial DNA | Molecular Characterization | ITS-rDNA |
| Pérez-Doria *et al.* | 2008 | *Lutzomyia* columbiana | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | RNAt - serine |
| Pérez-Doria *et al.* | 2008 | *Lutzomyia* tihuiliensis & *Lutzomyia* pia | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | RNAt - serine |
| Pérez-Doria *et al.* | 2008 | *Lutzomyia* hartmanni, *L. columbiana* & *L. tihuiliensis* | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | RNAt - serine |
| Absavaran *et al.* | 2009 | *Phlebotomus* Subgenus *Larroussius* | mitochondrial DNA sequences & ADNg | Taxonomic Identification & Barcoding | cyt b & EF Alfa |
| Boudabous *et al.* | 2009 | *P. chabaudi* & *P. riouxi* | mitochondrial DNA sequences &RFLP | Taxonomic Identification & Barcoding | COI |
| Florin *et al.* | 2010 | *L. shannoni* | mitochondrial DNA sequences & ADNg | Intraspecific Variability | COI & ITS2 |
| Khalid *et al.* | 2010 | *P. papatasi*, *P. bergeroti* & *P. duboscqi* | nuclear DNA sequences | Taxonomic Identification & Barcoding | ITS2 |
| Cohnstaedt *et al.* | 2011 | *Lutzomyia Verrucarum* group | mitochondrial DNA sequences | Phylogeny | COI |
| Belen *et al.* | 2011 | *P. papatasi*, *P. tobbi*, *P. sergenti*, | mitochondrial DNA sequences & ADNg | Genetic Structure | cyt b & ITS2 |
| Dvorak *et al.* | 2011 | *P. sergenti* | mitochondrial DNA sequences, ADNg & RAPD | Intraspecific Variability | ITS2, cyt b & RAPD markers |
| Hamarsheh *et al.* | 2011 | *P. papatasi* | Microsatellites | Molecular Characterization | PPEST Primers 1 to 40 |
| Latrofa *et al.* | 2011 | *P. perniciosus*, *P. perfiliewi*, *P. neglectus*, *P. papatasi*, & *Sergentomyia minuta* | mitochondrial DNA sequences & ADNg | Phylogeny | cyt b & ITS2 |
| Silva *et al.* | 2011 | *L. longipalpis* | RAPD | Genetic Structure | N/A |
| Pérez-Doria *et al.* | 2011 | *Lutzomyia* hartmanni | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | RNAt - serine |
| Latrofa *et al.* | 2012 | *P. perniciosus*, *P. perfiliewi*, *P. neglectus*, *P. papatasi*, & *Sergentomyia minuta* | RFLP | Taxonomic Identification & Barcoding | cyt b & ITS2 |
| Hoyos *et al.* | 2012 | *L. longipalpis* | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | COI |

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|---|---|---|---|---|---|
| Curler *et al.* | 2012 | Subfamilies *Psychodidae* | nuclear DNA sequences | Systematics | 18Sr |
| Scarpassa & Alencar | 2012 | *L. umbratilis* | mitochondrial DNA sequences | Speciation & Divergence | COI |
| Raja *et al.* | 2012 | *Phlebotomus papatasi* | mitochondrial DNA sequences | Intraspecific Variability | cytb |
| Kato *et al.* | 2012 | L. ayacuchensis | cDNA & protein sequences | Intraspecific Variability | RGD-containing peptide |
| Zapata *et al.* | 2012 | *Nyssomyia trapidoi* | mitochondrial DNA sequences & Isozymes | Genetic Structure | malate dehydrogenase, isocitrate dehydrogenase, glycerol-3-phosphate dehydrogenase, glucose-6-phosphate COI , cytb , dehydrogenase, hexokinase, phosphoglucomutas , fumarase & glucose phosphate isomerase, |
| Seblova *et al.* | 2013 | *Phlebotomus* orientalis | mitochondrial DNA sequences & RAPD | Intraspecific Variability | COI, cytb. RAPD primers: OPE16, OPI 12, 13, OPL5, OPO20 |
| Scarpassa & Alencar | 2013 | *Lutzomyia* umbratilis & *Lutzomyia anduzei* | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | COI |
| Contreras-Gutiérrez *et al.* | 2014 | 32 *Lutzomyia* species and 4 *Brumptomyia* species | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | COI |
| Maia *et al.* | 2015 | *Phlebotomus ariasi*, *P. perniciosus*, *P. sergenti* & *Sergentomiya minuta* | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | COI |
| Nzelu *et al.* | 2015 | 18 *Lutzomyia* species & *Warileya euniceae* | mitochondrial DNA sequences | Taxonomic Identification & Barcoding | COI |

**Table II. Primers (if applicable) used in the studies cited above, providing researchers with a list of primers to use in future research.**

| Article | Marker | Primer | | Sequence | |
|---|---|---|---|---|---|
| Essseghir *et al.*, 1997 | cytb-NADH1 | CB3-PDR | N1N-PDR | 5'-CA(T/C)ATTCAACC(A/T)GAATGATA-3' | 5'-GGTA(C/T)(A/T)TTGCCTCGA(T/A) TTCG(T/A)TATGA-3' |
| Depaquit *et al.*, 1998 | D2 (ADNr 28s) | C2' | D2' | 5'-GAAAAGAACTTTGRARAGAGA-3' | 5'-TCCGTGTTTCAAGACGGC-3' |
| Ishikawa *et al.*, 1999 | cytb | N1N | CB3 | 5'-GGCAYWTTGCCTCGAWTTCGWTATGA-3' | 5'-CAYATTCAACCWGAATGATA-3' |
| Aransay *et al.*, 2000 | 18S ADNr | F1 | R1 | 5'-GCGGTTGATYCTRCCAGT-3' | 5'-CYGCAGGTTCACCTACRG-3' |
| Di Muccio *et al.*, 2000 | ITS2 | 18S | 28S | 5'-CCTTTGTACACACCGCCCGT-3' | 5'-CCTTTGTACACACCGCCCGT-3' |
| Essseghir & Ready 2000 | ITS-rDNA | CB1-SE | CB3-R3A | 5'-TATCTACTACCCTGAGGACAAATATC-3' | 5'-GCTATTACTCCYCCTAACTTRTT-3' |
| Essseghir & Ready 2000 | ITS-rDNA | CB3-FC | N1N-FA | 5'-CAYATTCAACCWGAATGATA-3' | 5'-GGCAYWTTGCCTCGAWTTCGWTATGA-3' |
| Di Muccio *et al.*, 2000 | ITS2 | 5.8S | 28S | 5'-TGTGAACTGCAGGACACATG-3 | 5'-ATGCTTAAATTTAGGGGGTA-3' |
| Depaquit *et al.*, 2000 | D2 (ADNr 28s) | C2' | D2' | 5'-GAAAAGAACTTTGRARAGAGA-3' | 5'-TCCGTGTTTCAAGACGGC-3' |
| Depaquit *et al.*, 2000 | ITS2 | C1a | JTS3 | 5'-CCTGGTTAGTTTCTTTTCCTCCGCT-3' | 5'-CGCAGCTAACTGTGTGAAATC-3' |
| Uribe *et al.*, 2001 | ND4 | ND4ar | ND4c | 5'-AA(A/G)GCTCATGTTGAAGC-3' | 5'-ATTTAAAGG(T/C)AATCAATGTAA-3' |
| Bauzer *et al.*, 2002 (a & b) | Per | 5llper1 | 3llper1 | 5'-CAATGGCTTCTACATCACTC-3' | 5'-ACTTGCTGCTTCACTGTATC-3' |
| Lins *et al.*, 2002 | Cac | 5LIcac | 3LIcac | 5'-GTGGCCGAACATAATGTTAG-3' | 5'-CCACGAACAAGTTCAACATC-3' |
| Lins *et al.*, 2002 | Cac | cacdeg5D | cacdeg3B | 5'-TGYGCNACNGGNGARGCNTGG-3' | 5'-TAYTCRAARTTRTCCATDAT-3' |
| Arrivillaga *et al.*, 2002 | COI | CI-J-1632 | CI-N-2191 | 5'-TGATCAAATTTATAAT-3' | 5' -GGTAAAATTAAAATATAAACTTC-3' |
| Testa *et al.*, 2002 | cytb | CB1-SE | CB3-R3A | 5'-TATCTACTACCCTGAGGACAAATATC-3' | 5'-GCTATTACTCCYCCTAACTTRTT-3' |
| Testa *et al.*, 2002 | EF alfa | EF-F03 | EF-R04 | 5'-GCTCCTGGACATCGTGAYTT-3' | 5'-AGTGCTTCGTGGTGTATYTC-3' |
| Depaquit *et al.*, 2005 | ND4 | ND4ar | ND4c | 5'-AA(A/G)GCTCATGTTGAAGC-3' | 5'-ATTTAAAGG(T/C)AATCAATGTAA-3' |
| Parvizi & Ready 2006 | cytb | CB1-SE | CB3-R3A | 5'-TATCTACTACCCTGAGGACAAATATC-3' | 5'-GCTATTACTCCYCCTAACTTRTT-3' |
| Parvizi & Ready 2006 | cytb | N1N | CB3 | 5'-GGCAYWTTGCCTCGAWTTCGWTATGA-3' | 5'-CAYATTCAACCWGAATGATA-3' |
| Parvizi & Ready 2006 | cytb | CB1-SE | CB-R06 | 5'-TATCTACTACCCTGAGGACAAATATC-3' | 5'-TATCTAATGGTTTCAAAACAATTGC-3' |
| Mazzoni *et al.*, 2006 | Per | 5llper2 | 3llper2 | 5'-AGCATCCTTTTGTAGCAAAC-3' | 5'-TCAGATGAACTCTTGCTGTC-3' |
| Moin.Vaziri *et al.*, 2007 | cytb | N1N | CB3 | 5'-GGCAYWTTGCCTCGAWTTCGWTATGA-3' | 5'-CAYATTCAACCWGAATGATA-3' |
| Hamarsheh *et al.*, 2007 | cytb-NADH1 | CB3-PDR | N1N-PDR | 5'-CA(T/C)ATTCAACC(A/T)GAATGATA-3' | 5'-GGTA(C/T)(A/T)TTGCCTCGA(T/A) TTCG(T/A)TATGA-3' |
| Parvizi y Assmar 2007 | EF alfa | EF-F03 | EF-R04 | 5'-GCTCCTGGACATCGTGAYTT-3' | 5'-AGTGCTTCGTGGTGTATYTC-3' |
| Parvizi & Amirkhani 2008 | ITS-rDNA | CB1-SE | CB3-R3A | 5'-TATCTACTACCCTGAGGACAAATATC-3' | 5'-GCTATTACTCCYCCTAACTTRTT-3' |

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|---|---|---|---|---|---|
| Parvizi & Amirkhani 2008 | ITS-rDNA | CB3-FC | N1N-FA | 5'-CAYATTCAACCWGAATGATA-3' | 5'-GGCAYWTTGCCTCGAWTTCGWTATGA-3' |
| Parvizi & Amirkhani 2008 | ITS-rDNA | CB1-SE | CB-R06 | 5'-TATCTACTACCCTGAGGACAAATATC-3' | 5'-TATCTAATGGTTTCAAAACAATTGC-3' |
| Lins *et al.*, 2008 | paralytic | 5paralIdegC | 3paralIdegB | 5'-TGGAAYTTYACNGAYTT-3´ | 5'-TTRTTNGTRTCRTTRTC-3' |
| Lins *et al.*, 2008 | paralytic | 5IIpara2 | 3IIpara1 | 5'-ACGGACTTCATGCATTCATTC | 5'-TGGTGCTGATAAACTTGACG-3' |
| Mazzoni *et al.*, 2008 | Ca1D | 5LWIca1D | 3LWIca1D | 5'-CAGGATATAATGATGGATTG-3' | 5'-CACGAACAAGTTGATAAT-3' |
| Mazzoni *et al.*, 2008 | Cac | 5LIcac | 3LIcac | 5'-GTGGCCGAACATAATGTTAG-3' | 5'-CCACGAACAAGTTCAACATC-3' |
| Coutinho-Abreu *et al.*, 2008 | cytb | N1N | CB3 | 5'-GGCAYWTTGCCTCGAWTTCGWTATGA-3' | 5'-CAYATTCAACCWGAATGATA-3' |
| Depaquit *et al.*, 2008 | ITS2 | C1a | JTS3 | 5'-CCTGGTTAGTTTCTTTTCCTCCGCT-3' | 5'-CGCAGCTAACTGTGTGAAATC-3' |
| Depaquit *et al.*, 2008 | ND4 | ND4ar | ND4c | 5'-AA(A/G)GCTCATGTTGAAGC-3' | 5'-ATTTAAAGG(T/C)AATCAATGTAA-3' |
| Mazzoni *et al.*, 2008 | Per | 5IIper2 | 3IIper2 | 5'-AGCATCCTTTTGTAGCAAAC-3' | 5'-TCAGATGAACTCTTGCTGTC-3' |
| Mazzoni *et al.*, 2008 | Rp49 | 5RP49semideg1 | 3IIRP49exp2 | 5'-TTCATYCGYCAYCAGWSBGA-3' | 5'-GGGCGATCTCAGCACAGTAT-3' |
| Mazzoni *et al.*, 2008 | RpL17A | 5LLrpL17A | 3LLrpL17A | 5'-TCAATTGCGCCGACAATAC-3' | 5'-GCTGATCCTTTCATTTCGCC-3' |
| Mazzoni *et al.*, 2008 | RpL36 | 5LWIrpL36 | 3LWIrpL36 | 5'-GTTCCTCACGCTTCCTCTTG-3' | 5'-AAAGTGAAAGGACTCCGCCC-3' |
| Mazzoni *et al.*, 2008 | RpS19a | 5LWrpS19 | 3LWrpS19 | 5'-TGATCAACACAAGATTGTCCG-3' | 5'-ACACCATTCCTCTTACGACC-3' |
| Mazzoni *et al.*, 2008 | TfIIA-L | 5LLTfIIA-L | 3LLTfIIA-L | 5'-GATAATGATCCAGACGATGCC-3' | 5'-GAAAACATAGTCCTTCCCACC-3' |
| Mazzoni *et al.*, 2008 | Up | 5LLup | 3LLup | 5'-GCAACAAGTCCAAAGAGCAG-3' | 5'-TCATAGGAGCGGGTGTCAAC-3' |
| Mazzoni *et al.*, 2008 | zetacop | 5LLzetacop | 3LLzetacop | 5'-GGATGCAGATCCTTCATCCG-3' | 5'-CGACCACTTCAGTTGTTCTC-3' |
| Pérez-Doria *et al.*, 2008 (a, b & c) | RNAt - serine | N/A | N/A | 5'-CA(T/C)ATTCAACC(A/T)GAATGATA-3' | 5'-GGTA(C/T)(A/T)TTGCCTCGA(T/A) TTCG(T/A)TATGA-3' |
| Boudabous *et al.*, 2009 | COI | LepF | LepR | 5'-ATTCAACCAATCATAAAGATATTGG-3' | 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' |
| Boudabous *et al.*, 2009 | ITS-rDNA | CB1-SE | CB3-R3A | 5'-TATCTACTACCCTGAGGACAAATATC-3' | 5'-GCTATTACTCCYCCTAACTTRTT-3' |
| Boudabous *et al.*, 2009 | ITS-rDNA | CB3-FC | N1N-FA | 5'-CAYATTCAACCWGAATGATA-3' | 5'-GGCAYWTTGCCTCGAWTTCGWTATGA-3' |
| Absavaran *et al.*, 2009 | cytb | CB3-FC | CB-R06 | 5'-CAYATTCAACCWGAATGATA-3' | 5'-TATCTAATGGTTTCAAAACAATTGC-3' |
| Absavaran *et al.*, 2009 | EF-1 alpha | EF-F05 | EF-F08 | 5'-CCTGGACATCGTGATTTCAT-3' | 5'-CCACCAATCTTGTAGACATCCTG-3' |
| Florin *et al.*, 2010 | COI | | | Primer sequence nor citation provided. | |
| Florin *et al.*, 2010 | ITS2 | | | Primer sequence nor citation provided. | |
| Khalid *et al.*, 2010 | ITS2 | C1a | JTS4 | 5'-CCTGGTTAGTTTCTTTTCCTCCGCT-3' | 5'-TGCAGCTAACTGTGTGAAAT-3' |
| Belen *et al.*, 2011 | ITS2 | C1a | JTS3 | 5'-CCTGGTTAGTTTCTTTTCCTCCGCT-3' | 5'-CGCAGCTAACTGTGTGAAATC-3' |
| Belen *et al.*, 2011 | cytb | CB1-SE | CB-R06 | 5'-TATCTACTACCCTGAGGACAAATATC-3' | 5'-TATCTAATGGTTTCAAAACAATTGC-3' |
| Latrofa *et al.*, 2011 | cytb and nd1 | PhleF | PhleR | 5'-AATAAATTAGGAGGAGTAATTGC-3' | 5'-TCGAWTTCGWTTATGAT AA T-' |

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| | | | | | |
|---|---|---|---|---|---|
| Latrofa *et al.*, 2011 | ITS2 | C1a | JTS3 | 5'-CCTGGTTAGTTTCTTTTCCTCCGCT-3' | 5'-CGCAGCTAACTGTGTGAAATC-3' |
| Cohnstaedt *et al.*, 2011 | COI | LCOI490 | HCO2198 | 5'-GGTCAACAAATCATAAAGATATTGG-3' | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' |
| Silva *et al.*, 2011 | RAPD primers | Opa-3 | Opa-4 | 5'-AGTCAGCCAC-3' | 5'-AATCGGGCTG-3' |
| Silva *et al.*, 2011 | RAPD primers | Opa-9 | Opa-15 | 5'-GGGTAACGCC-3' | 5'-TTCCGAACCC-3' |
| Dvorak *et al.*, 2011 | ITS2 | C1a | JTS3 | 5'-CCTGGTTAGTTTCTTTTCCTCCGCT-3' | 5'-CGCAGCTAACTGTGTGAAATC-3' |
| Dvorak *et al.*, 2011 | cytb | VD-F | VD-R | 5'-TATGTACTACCATGAGGACAAATATC-3' | 5'-TAAAAGGGGCTTCAACTGGA-3' |
| Pérez-Doria *et al.*, 2011 | RNAt - serine | N/A | N/A | 5'-CA(T/C)ATTCAACC(A/T)GAATGATA-3' | 5'-GGTA(C/T)(A/T)TTGCCTCGA(T/A) TTCG(T/A)TATGA-3' |
| Hoyos *et al.*, 2012 | COI | LCO1490 | HCO2198 | 5' -GGTCAACAAATCATAAAGATATTGG - 3' | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' |
| Kato *et al.*, 2012 | RGD-containing peptide | PT2F1 | PT2R1 | 5'-AAGTACTCTAGCAATTGTGAGC-3' | 5'-CTCTTCGCTATTACGCCAGCTG-3' |
| Scarpassa y Alencar 2012 | COI | UEA3 | UEA10 | 5'-TATAGCATTCCCACGAATAAATAA-3' | 5'-TCCAATGCACTAATCTGCCATATTA-3' |
| Curler *et al.*, 2012 | 18S ADNr and Peregrin | | | Primer sequence nor citation provided. | |
| Zapata *et al.*, 2012 | COI | LepF | LepR | 5'-ATTCAACCAATCATAAAGATATTGG-3' | 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' |
| Zapata *et al.*, 2012 | COI | CB3-PDR | N1N-PDR | 5'-CA(T/C)ATTCAACC(A/T)GAATGATA-3' | 5'-GGTA(C/T)(A/T)TTGCCTCGA(T/A) TTCG(T/A)TATGA-3' |
| Boudabous *et al.*, 2012 | COI | CB3-PDR | N1N-PDR | 5'-CA(T/C)ATTCAACC(A/T)GAATGATA-3' | 5'-GGTA(C/T)(A/T)TTGCCTCGA(T/A) TTCG(T/A)TATGA-3' |
| Latrofa *et al.*, 2012 | cytb and nd1 | PhleF | PhleR | 5'-AATAAATTAGGAGGAGTAATTGC-3' | 5'-TCGAWTTCGWTTATGAT AA T-3' |
| Latrofa *et al.*, 2012 | ITS2 | C1a | JTS3 | 5'-CCTGGTTAGTTTCTTTTCCTCCGCT-3' | 5'-CGCAGCTAACTGTGTGAAATC-3' |
| Scarpassa & Alencar 2013 | COI | UEA3 | UEA10 | 5'-TATAGCATTCCCACGAATAAATAA-3' | 5'-TCCAATGCACTAATCTGCCATATTA-3' |
| Seblova *et al.*, 2013 | COI | LepF | LepR | 5'-ATTCAACCAATCATAAAGATATTGG-3' | 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' |
| Seblova *et al.*, 2013 | cytb | VD-F | VD-R | 5'-TATGTACTACCATGAGGACAAATATC-3' | 5'-TAAAAGGGGCTTCAACTGGA-3' |
| Scarpassa & Alencar 2013 | COI | LCO1490 | HCO2198 | 5' -GGTCAACAAATCATAAAGATATTGG - 3' | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' |
| Contreras-Gutiérrez *et al.*,2014 | COI | LCO1490 | HCO2198 | 5' -GGTCAACAAATCATAAAGATATTGG - 3' | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' |
| Maia *et al.*, 2015 | COI | LCO1490 | HCO2198 | 5' -GGTCAACAAATCATAAAGATATTGG - 3' | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' |
| Nzelu *et al.*, 2015 | COI | LCO1490 | HCO2198 | 5' -GGTCAACAAATCATAAAGATATTGG - 3' | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' |