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# CRITICAL ASPECTS OF DNA-BASED METHODS FOR ERIOPHYOID MITE DIAGNOSTICS AND GENETIC STUDIES: REVIEW, PROSPECTS AND CHALLENGES

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## Abstract

DNA-based methods have revolutionized the field of species diagnostics, and today it has applications for an increasingly number of taxa including several Acari. The media excitement around the international Barcode project is an example. Besides their potential for species identification, DNA marker techniques are nowadays routinely being also used for addressing ecological, evolutionary, phylogenetic and genetic questions. In contrast to other groups of plant mites and despite the economical relevance of many species of Eriophyoidea, very few scientists have dared so far to use DNA methods for the study of this group of mites; their very small size being certainly the major cause. However, DNA-based techniques are now well established and their advantages as well as limitations have been realized. We review here the main techniques used for identification and discus on their applicability in eriophyoids. Main results from the literature will be examined. We will emphasize prospects and challenges of the molecular genetics approach to study several essential issues of the eriophyoid biology to: clarify suspect synonymies, test hypothesis of cryptic species; examine the occurrence of biotypes, especially in rapport to virus ability; understand colonization patterns of invasive species; and use of biological control agents against invasive plants. We will discuss these questions that can be link to economical issues, together with more fundamental aspects as reviewing the phylogeny of the Eriophyoidea. Much is now expected from molecular techniques in many fields of biology. Eriophyoids should not be the exception.

## Keywords

Molecular markers, species diagnostics, phylogeny, Eriophyoidea

## Introduction

DNA-based techniques are increasingly used in Acarology studies, particularly for systematic and population biology, having contributed to explore some questions that were difficult to answer some years. The field of biological diagnostics, for instance, was revolutionized with the advent of the polymerase chain reaction (PCR). The ability to amplify numerous copies of a gene or genomic region of interest opened up a world of possibilities in terms of identification of organisms, genes, genotypes, mutations and populations. Hence, besides providing solid taxonomic criteria, data obtained through DNA based analyses can aid in testing phylogenetic hypotheses and gain in understanding the partition of the variability within a species.

Although efforts in using molecular biology techniques have been also made on Eriophyidae, the attempts are still scarce compared to other plant mite families. This is a regrettable observation, considering that among plant feeding

mites, Eriophyidae represent the second group in economic importance as pests after the Tetranychidae (Lindquist and Amrine 1996) and that is one with the higher number of taxa (3.440 species from 301 genera) (Amrine Jr. and De Lillo 2003). Although Eriophyoidea is an extensively studied group, studies have mainly focused on the biology and control of a limited number of species basically based on observational data (Lindquist et al 1996; Davies et al 2001). In the last few years, the wealth of DNA-based resources has started to be used in eriophyoids. This paper presents a review of the advances made using molecular techniques and gives prospects and challenges to be addressed in the near future.

## Which molecular genetic marker to use?

In the last two decades, several key advances in molecular genetics appeared which have greatly increased the impact of molecular techniques on biology. Most important have been: (1) the development of PCR, which amplifies specified stretches of DNA to useable concentrations; (2) the application of evolutionarily conserved sets of PCR primers (Simon et al. 1994); (3) the advent of hypervariable microsatellite loci (Goldstein and Schlötterer 1999); and (4) the advent of routine DNA sequencing in biology laboratories. These innovations, coupled with the recent explosion of powerful analyses and relatively user-friendly computer programs (Excoffier and Heckel 2006), made that much of the power inherent in molecular genetic data can be useable for biological studies.

After the invention of the PCR technology, a large number of approaches to generate molecular markers have been created (Behura 2006). These techniques are well established and their advantages as well as limitation have been realized. All genetic markers reflect differences in DNA sequences, usually with a trade-off between precision and convenience, and then in addition to technical details, focusing on important properties helps to make sense of the methods. In addition, separate loci can provide independent test of hypothesis, thus using several together can increase sensitivity. Table 1 summarizes attributes of markers commonly used. Among them, the media excitement around the international Barcode project is an example of the power and limitations of using molecular techniques, in this case for species diagnostics purposes (Savolainen et al. 2005).

## The Barcode initiative

The concept of a DNA barcode has recently been proposed as a method of diagnosing species both known and unknown. The DNA barcode approach uses nucleotide sequences consisting of unique combinations of bases occurring in conserved regions of genes that are easily amplified with PCR and direct sequencing. It uses short DNA sequence from a standardized and agreed-upon position in the genome for molecular diagnosis and identification at the species level. For most animals, including the Acari, the Cytochrome C Oxidase subunit 1 (*COI*) mitochondrial gene has become the standard barcode region.

## When eriophyid biology and molecular techniques meet:

As for other Acari, the Eriophyidae have beneficiate of the rapid development of molecular methods that measure genetic variation. Although the palette of technical approaches used for this group is still limited, important advances could be done by using DNA-based techniques.

The nuclear regions used in eriophyoids include the ribosomal Internal Transcribed Spacer (ITSI and ITS2) and associated genes (18S, 5.8 and 28S). Results obtained with *Cecidophypsis* mites (Kumar et al. 1999; Lemmetty et al. 2001) have indicated that the ITSI was more useful than ITS2 to distinguish closely related species. In addition, microsatellite loci have been used by (Carew et al. 2004) to evaluate population structure of a grapevine pest *Colomerus vitis* (Pagenstecher). Among the mitochondrial genes, the mitochondrial 16S was used (Navia et al., 2005), and the COI of four eriophyid – *Aceria tulipae* (Keifer), *Aceria eximia* Sukhareva, *Eriophyes pyri* (Pagenstecher) and *Floracarus perrepae* Knihinicki & Boczek– has been already sequenced (data published on data bases only; source www.ncbi.nlm.nih.gov, on May, 18th 2008).

## Identification of species

Molecular tools can be extremely useful in Eriophyoidea systematics considering that using exclusively morphological characters to their identification present several limitations. Because of the considerable reduction and simplification in the body plan of eriophyoids, the structures that can be used for eriophyoid systematics are scarce, compared to most of other mites. Another limitation of some species is their lack of ontogenetic diversity as well as the lack of useful

characters peculiar to the adult male (Lindquist and Amrine 1996). Advances in molecular biology have provided data on nucleotide variation that added to more traditional morphological features help in establishing reliable criteria to determine species.

**Table 1.** Comparison of features of frequently used molecular marker techniques. Abbreviations of each marker appear in the table and the corresponding full name is indicated at the bottom.

| | Abundance | Reproducibility | Single locus | Degree of polymorphism | Codominant | Technical requirement | Tissue required | PCR assay |
|---|---|---|---|---|---|---|---|---|
| ***Mitochondrial*** | | | | | | | | |
| RFLP | high | straight | yes | low to high | yes | high | high | yes |
| Sequences | high | straight | yes | medium | yes | medium | low | no*** |
| ***Multilocus nuclear*** | | | | | | | | |
| RAPD | high | limited | no | high | no | low | low | yes |
| AFLP | high | limited | no | high | no | medium | medium | yes |
| ***Single-locus nuclear*** | | | | | | | | |
| Allozymes | low | straight | yes | low | infrequent | medium | high** | no |
| Microsatellites | high | indirect | yes | high | yes | high | low | yes |
| Anonymous scn | high | indirect | yes | medium | yes | medium | low | yes |
| Specific scn | medium | straight | yes | low | yes | medium | low | yes |
| ribosomal DNA | low | straight | * | medium | yes | medium | low | yes |

Full name of markers: RFLP restriction fragment length polymorphism; RAPD random amplified polymorphic DNA; AFLP amplified fragment length polymorphism; *scn* single copy nuclear.

* ribosomal DNA consists of tandem arrays of a few regions. In some taxa the arrays are effectively identical and regions act as single loci, but in some taxa there can be many different sequences within individuals, in which case rDNA acts more like a multilocus system
** Fresh or frozen material is needed for allozymes. By contrast, all other techniques allow using ethanol preserved samples
***Sequencing it self do not use PCR techniques but sequences are usually obtained after PCR amplification of the targeted DNA fragment.

A series of studies on species identification, phylogeny and intraspecific variability in the *Cecidophyopsis* Keifer genus, have been conducted since 1995 (Fenton et al. 1995; Fenton et al. 1996; Fenton et al. 1997; Fenton et al. 2000). This group includes mite species known to occur on twelve *Ribes* species and several of them are serious agricultural pest(De Lillo and Duso 1996). Kumar and co-workers (1999) developed a PCR multiplex technique for identifying *Cecidophyopsis* mites using species-specific differences in ITS-1 sequences. The PCR multiplex technique presented in Kumar and coworkers (2001) was used by (Lemmetty et al. 2001) to conduct a detailed study on the identification of *Cecidophypsis* mites on *Ribes* in Finland.

Grapevine eriophyoid mites – *Colomerus vitis* Pagenstecher and *Calepitrimerus vitis* Nalepa – are recognized pests. The identity of these mites has recently been investigated using molecular markers - PCR-RFLP of the ITS-1 and microsatellite (Carew et al. 2004). Authors concluded that PCR-RFLP of the ITS-1 region could be routinely used as a rapid diagnostic tool for confirming the species of mite present in a vineyard.

## Plant-mite interaction

One very useful application of genetic data in pest management is to investigate specialisation of mites to their host plant, which in some cases have uncovered host races. In eriophyoids, some economically important issues could be addressed by using DNA markers as illustrated below.

The eriophyid mite *Aceria cajani* (Channabasavanna) is the vector of the agent of pigeonpea sterility mosaic disease (PSMD).

Integrated management of PSMD includes the development of resistant cultivars. However, pigeonpea genotypes resistance was found to be location specific. It is possible that the breakdown in PSMD resistance at various locations is due to the occurrence of different *Aceria* species or biotypes of *A. cajani*. Aiming to test this hypothesis, the variation of *A. cajani* was assessed using the ITS region and associated rDNA genes, by analyzing nucleotide sequences and patterns of restriction enzymes (Kumar et al. 2001). Results strongly suggested that *A. cajani* on pigeonpea across the Indian subcontinent constitutes a single species.

The phylogenetic relationship of seven species of *Cecidophyopsis* mites with its *Ribes* hosts was inferred from ribosomal sequences of the ITS region and surrounding regions (18S, 5.8S and 28S) (Fenton et al. 2000). The comparison of two phylogenetic trees (mites versus hosts) showed clear differences of structure, implying that the mite speciation did not closely follow speciation events in the plant hosts.

## Pest-movements, colonisation patterns and bioinvasions: the coconut mite *Aceria guerreronis*, a case study

The coconut mite, *Aceria guerreronis* Keifer, has recently spread and rapidly established in the main coconut production areas worldwide, being considered as an invasive species. The mite has not been recorded in the Indo-Pacific region, the area of origin of coconut, suggesting that it has infested coconut only recently. To investigate the geographical origin, ancestral host associations, and colonization history of the mite (Navia et al. 2005) conducted a phylogeography study, using DNA sequence data from two mitochondrial (16 S) and one nuclear region (ITS1 and ITS2) from samples from the Americas, Africa and the Indo-ocean region. The results suggest that the mite originates from the Americas and not from the ancestral region of coconut in South East Asia and lend evidence to a previous hypothesis that the original host of the mite is a non-coconut palm (Fig 1).

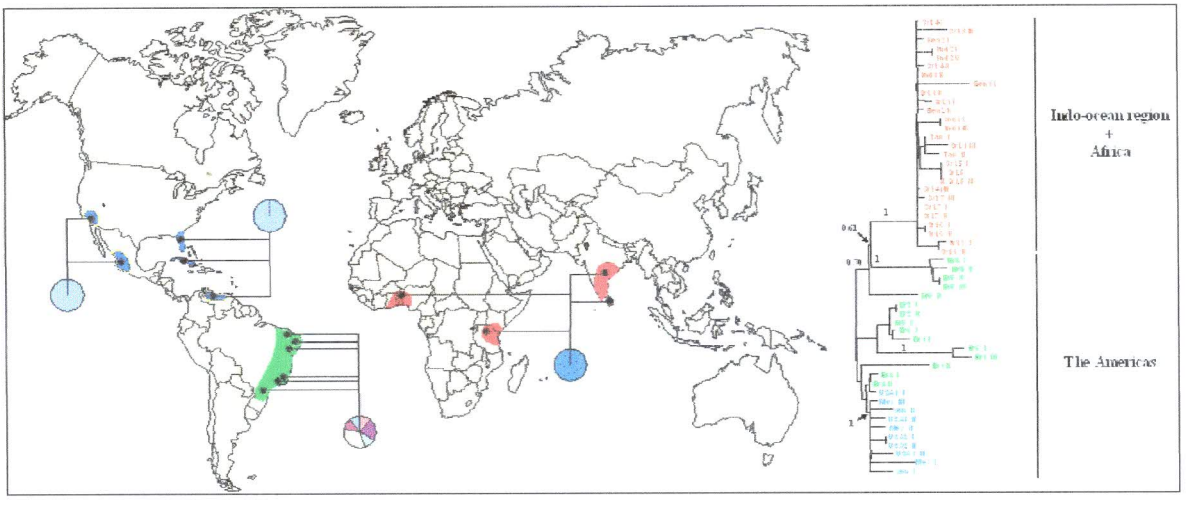


**Figure 1**. Phylogeographical history of the coconut mite, *Aceria guerreronis*. The three geographical regions sampled are indicated in red (Indo-ocean region), green (Brazil) and blue (other American countries) (a) different mitochondrial haplotypes detected and their frequency in the different sampled localities (black dots) are indicated by the pie charts. The highest nucleotide diversity was found in Brazil where six out of the seven haplotypes were present. By contrasts one haplotype (here in pink) was found in Central and North America and a single one (here in yellow) was shared by non-American mites from Africa and the Indo-ocean region (India and Sri-Lanka). (b) Congruently, the tree constructed with the nuclear ITS sequences revealed that all non-American samples (in red) are very little diversified and cluster together, whereas the Brazilian (in green) are represented in several branches of the tree. The rest of the American samples (in blue) are gathered in a single cluster.

## Molecular techniques to study eriophyids: challenges and new avenues

The field of molecular marker technology is fast progressing by adopting new forms and innovative approaches of the existing genetic principles in detecting DNA polymorphism and the minute eriophyoids in increasingly beneficiating of all this progress. Much is now expected of the molecular techniques on eriophyoids biology. We discuss some of the major issues that we think might take advantage of molecular approaches in the next future. Much progress might be expected for systematis. The majority of taxonomic groupings of eriophyoid species are artificial. As a result, the current classification has little predictive power (Lindquist and Amrine 1996). The lack of information on the Eriophyoidea phylogeny has been an important limitation on the progress of systematic and biology of the group. For lower taxonomic levels, uncertainties on Eriophyoidea systematics are also numerous and molecular techniques can answer questions on synonymies and cryptic species. For more applied areas, molecular studies can significantly contribute to define pest management strategies in eriophyoid, as for example by using them as weed natural control agents or for they relevance as phytovirus vectors. Among phytophagous mites, eriophyoid are becoming increasingly recognized for their potential as invasive. Molecular data can provide information on the routes of colonization or pathways of invasive eriphyoid, required to guide adoption of quarantine measures.

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