Exp Appl Acarol (2010) 51:257–271
DOI 10.1007/s10493-009-9301-z

DNA-based methods for eriophyoid mite studies: review, critical aspects, prospects and challenges

Maria Navajas · Denise Navia

Received: 17 April 2009 / Accepted: 19 August 2009 / Published online: 14 October 2009

Abstract Besides their potential for species identification, DNA-based methods are also routinely used for addressing ecological, evolutionary, phylogenetic and genetic questions to study several groups of Acari. However, in contrast to other plant-feeding 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 certainly has influenced this. In this review we examine the main techniques that have been used to study eriophyoid mites and discuss the results from the literature where DNA methods have provided significant advances to address several essential questions of the eriophyoid biology, e.g., to clarify suspect synonymies, to test hypothesis of cryptic species, to examine the occurrence of biotypes, especially in relation to virus ability or host-plant associations, to understand colonization patterns of invasive species, and for uses as biological control agents against invasive plants. We discuss these questions which might be related to agricultural issues, together with more fundamental aspects as the revision of the phylogeny of the Eriophyoidea. We discuss on the advantages as well as limitations of the most commonly used genetic markers and emphasize prospects and challenges of new molecular approaches. Much is now expected from molecular techniques in many fields of biology and for virtually all taxa. Eriophyoids should not be the exception.

Keywords Eriophyoidea · DNA · Molecular systematics · Phylogeny · Cryptic species · Pest management · Invasive species

M. Navajas
INRA, UMR CBGP (INRA/IRD/CIRAD/Montpellier SupAgro), Campus International de Baillarguet, CS 30016 34988 Montferrier sur Lez, France
e-mail: navajas@supagro.inra.fr

D. Navia (✉)
Laboratory of Plant Quarantine, Embrapa Recursos Genéticos e Biotecnologia, Postal Code 02372, 70.770-900 Brasília, DF, Brazil
e-mail: navia@cenargen.embrapa.br

Introduction

DNA-based methods have revolutionized many fields of biology, and nowadays are currently used in an increasingly number of taxa to address a wide diversity of questions. With the advent of the PCR (1987), the ability to amplify numerous copies of a gene or genomic region of interest opened a world of possibilities not only in terms of identification of organisms, genes and genotypes, but also, data obtained through DNA based analyses can aid to address ecological and evolutionary questions. Genetic markers reflect differences in DNA sequences and are used to provide raw information to make estimates of genetic diversity at all taxonomic levels revealing otherwise unobtainable information of the relationships among taxonomical units (Ben-Ali et al. 2000; Navajas and Fenton 2000; Behura 2006). The utility of molecular data to provide solid taxonomic criteria is now well established, being widely used to accurately differentiate between species (Caterino et al. 2000; Tautz et al. 2003). For population studies, molecular information allows estimating gene flow, identifying haplotypes and lineages or predicting migration and colonization history (Sunnucks 2000; Garant and Kruuk 2005). Molecular methods are also of great interest for testing phylogenetic hypotheses, providing the means to propose solid, statistically supported, phylogenies.

As for other Acari, the Eriophyoidea has also benefit of the rapid development of molecular methods. However the attempts are still scarce compared to other plant mite groups. This is a regrettable observation, because among the plant feeding mites, Eriophyidae represent the second family economically important as crop pests after the Tetranychidae (Lindquist and Amrine 1996). It is also the family with the highest number of taxa (about 3,700 species from 350 genera) (Amrine and De Lillo 2006). DNA-based resources have started to be used in eriophyids about 15 years ago, with the first publications dated from 1995. Although the palette of technical approaches used for this group is still limited, important advances have been done using DNA-based techniques, which had contributed to explore some questions that were difficult to answer some years ago. This paper presents a brief introduction on the use of molecular techniques and their evolution through time, together with a review on the advances they have permitted in the study of eriophyids. Prospects and challenges of the DNA-based methods that have the potential to be used for eriophyoid mite knowledge in the near future are discussed.

Advances in molecular genetics and the use of DNA-based markers

Several key advances in molecular genetics appeared in the last two decades, which have greatly increased the impact of molecular techniques in biology in general. Among the most important are: (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; Ben-Ali et al. 2000; Navajas and Fenton 2000); (3) the advent of hypervariable microsatellite loci (Goldstein and Schlötterer 1999); and (4) the advent of routine DNA sequencing in biology laboratories and technical platforms. These innovations, coupled with the recent explosion of powerful analyses and relatively user-friendly computer programs (Excoffier and Heckel 2006), bring about that much of the power intrinsic to molecular genetic data became accessible for non specialists. A number of informative review papers describing the use of molecular techniques in natural populations have appeared in the recent literature (Sunnucks 2000; DeYoung and Honeycutt 2005; Behura 2006; Gariepy et al. 2007; Cusson 2008). The field continuously progresses

expanding the sequencing capacities at lower costs and miniaturizing the amounts of DNA template. A quantum leap has been done with the advent of the so-called next-generation DNA sequencing (NGS) methods which has the potential to dramatically accelerate biological research (Shendure and Ji 2008). These rapidly progressing technologies increase the speed and capacity of sequencing by 100 to 100,000 fold from current methods and can generate more than a billion bases of data in a single run.

The use of molecular markers is often based on a trade-off between precision and convenience. These techniques are well established and their advantages as well as limitations have been realized, and are summarized in Table 1 for the most commonly used markers. Focusing on important properties of the molecular markers helps to sense making of the methods used (Behura 2006; Cusson 2008). Concerns on their application exist which need to be understood for sense making interpretation of data derived from their use in some situations (Nichols 2001). As an example, Navajas and Boursot (2003) showed that whereas a nuclear marker, the ribosomal ITS2 region, was diagnostic to separate two closely but well defined spider mite species, *Tetranychus urticae* Koch and *T. turkestani* Ugarov & Nikolskii, the same two species were polyphyletic based on DNA sequences obtained with a second marker defined in the mitochondrial genome. Such discrepancies reflect that phylogenetic trees based on genes and true species phylogenies, or species trees might not be the same (Galtier and Daubin 2008). The time back to the branching points, and even the branching order, can be different between the gene tree and the species tree (Nichols 2001; Edwards 2009), which must be taken into account when estimating the speciation events, especially the more recent branches, to avoid inaccurate definition of species. Because separate loci can provide independent test of hypothesis, using several together increases sensitivity. The possibility to use multi-loci approaches follows the advances in molecular techniques (Rodrigo et al. 2008) and is strongly recommended to infer solid phylogenies.

The DNA- Barcoding initiative

One significant application of molecular methods is for species identification. The molecular diagnostics of species has raised much interest in practically all groups of organisms. The excitement around the international Barcode project (Savolainen et al. 2005) is an example. The concept of a DNA barcode has been proposed as a method of diagnosing species, which uses short DNA sequences consisting of unique combinations of bases occurring in conserved regions of genes that are easily amplified with PCR and direct sequencing. For most animals, including the Acari, the cytochrome c oxidase subunit 1 mitochondrial gene (COI) has become the standard barcode region. While popular for molecular diagnostics, several of the limits of barcode uses, which are mainly inherent to mtDNA features, are now well known and should be taken into account when using the barcode approach (Darling and Blum 2007). While there is no doubt that molecular data is useful for species identification, the need in maintenance of the associated morphological information to a barcode not represent a consensus. DNA barcode cannot replace morphology for identification and classification but the two approaches should be synergistically used (Tautz et al. 2003; DeSalle et al. 2005). While DNA barcoding methods were started to be used for species identification in spider mites (Tetranychidae) (Ben-David et al. 2007; Carbonnelle et al. 2007; Hinomoto et al. 2007), the approach has not yet been used for eriophyoid mites. Eriophyid researches will certainly beneficiate from studies on barcoding conducted on other mite groups or even other groups of organisms.

Table 1 Comparison of features of frequently used molecular marker techniques for molecular population biology studies

	Abundance	Reproducibility	Single locus	Degree of polymorphism	Codominant	Technical requirement	Material required	PCR assay	Rapid transfer to new taxa
Mitochondrial									
RFLP	High	Straight	Yes	Low to high	Yes	High	High	Yes	Yes
Sequences	High	Straight	Yes	Medium	Yes	Medium	Low	No[c]	Yes
Multilocus nuclear									
RAPD	High	Limited	No	High	No	Low	Low	Yes	Yes[d]
AFLP	High	Limited	No	High	No	Medium	Medium	Yes	Yes
Single-locus nuclear									
Allozymes	Low	Straight	Yes	Low	Infrequent	Medium	High[b]	No	Yes
Microsatellites	High	Indirect	Yes	High	Yes	High	Low	Yes	Medium
Anonymous scn[e]	High	Indirect	Yes	Medium	Yes	Medium	Low	Yes	Medium[f]
Specific scn	Medium	Straight	Yes	Low	Yes	Medium	Low	Yes	Medium[f]
Ribosomal DNA	Low	Straight	*de facto*[a]	Medium	Yes	Medium	Low	Yes	Yes

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

[a] 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

[b] Fresh or frozen material is needed for allozymes. By contrast, all other techniques allow using ethanol preserved samples

[c] Sequences it self do not use PCR techniques but they are usually obtained after PCR amplification of the targeted DNA fragment

[d] Transfer of experimental conditions some times difficult because of poor reproducibility among batches of experiences or laboratories

[e] Cloned single-copy nuclear (scn) is non-repetitive nuclear sequences that occur with a frequency of one per haploid genome

[f] Lack of extensive research on this type of markers

When eriophyoid biology and molecular techniques meet

The wealth of resources in molecular genetics are still poorly used to study eriophyids in comparison with other main families of plant mites (Fig. 1). A bibliographic review on studies on plant mites involving molecular tools published during the last 20 years showed a high difference on the number of papers on Tetranychidae (46) and on Eriophyidae (11), which are respectively the first and the second economically important mite families (Fig. 1). A high number of publications on the predator family Phytoseiidae (31) was also found. This family includes the major biological control organisms used against crop mite pests. Likewise, the number of nucleotide sequences deposited in GenBank until March 2009 was significantly lower in Eriophyidae (207) than in Tetranychidae (725). At present, eriophyid nucleotide sequences deposited in GenBank belong to a reduced number of species: 21 species from six genera—*Aceria*, *Calepitrimerus*, *Cecidophyopsis*, *Colomerus*, *Eriophyes* and *Floracarus* (www.ncbi.nlm.nih.gov accessed on 28th March 2009). All these genera are included in the family Eriophyidae. There is no information available on Phytoptidae or Diptilomiopidae mites. Although these two last families are not of economic importance, their phylogenic position among the Eriophyoidea, gained by molecular information, would be of interest for a comprehensive study of the group.

The molecular markers used so far to study eriophyids are the same commonly used in other groups of mites (see Table 2). The nuclear regions include the ribosomal Internal Transcribed Spacers (ITS1 and ITS2) and associated genes (18S, 5.8 and 28S). Using these genomic regions in *Cecidophyopsis* mites (Kumar et al. 1999; Lemmetty et al. 2001) showed that the ITS1 was more informative than the ITS2 to distinguish between closely related species. Among mitochondrial genes, the 16S (the small unit of the mitochondrial ribosomal gene) has been used only to study intraspecific variation of *Aceria guerreronis* Keifer (Navia et al. 2005) and to detect the occurrence of a complex of species in the *Aceria tosichella* Keifer taxon (Carew et al. 2004). In addition, sequences of fragments of the COI of four eriophyid species—*Aceria tulipae* Keifer, *Aceria eximia* Sukhareva, *Eriophyes pyri* (Nalepa) and *Floracarus perrepae* Knihinicki & Boczek—are available (data published on data bases only; source www.ncbi.nlm.nih.gov on 28th March 2009). Among nuclear markers, the D2 region (Divergent region 2) of the large subunit ribosomal DNA, has been reported as having the potential for species identification (Sonnenberg et al. 2007). It was successfully used to investigate intraspecific variation and identify genotypes of the eriophyid *F. perrepae* (Goolsby et al. 2006) and on the occurrence of a complex of species of the *Abacarus hystrix* (Nalepa) taxon (Skoracka and Dabert in press). Also the nuclear Adenine Nucleotide Translocase (*ANT*) gene was evaluated to detect cryptic

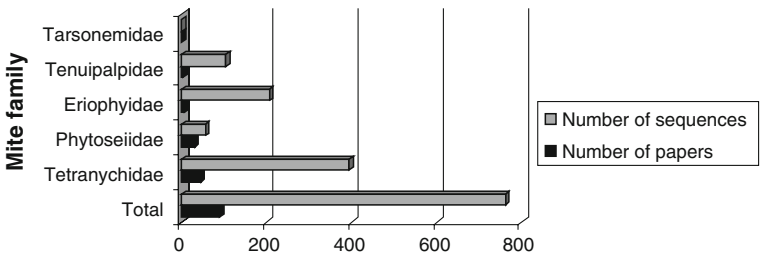

Fig. 1 Contribution of DNA data to the study of several plant mite families, estimated in number of published papers (CAB 1990–2009 database) and number of DNA sequences submitted to GenBank (on March 28th, 2009)

Table 2 PCR-based assays developed for eriophyoid mites

Eriophyoid mite	Region	Primary application	Reference
Cecidophyopsis alpina, *C. aurea*, *C. grossulariae*, *C. ribis*, *C. selachodon*, *C. spicata*, *Phyllocoptes gracilis*	ITS 1	Identification	Fenton et al. 1995
Cecidophyopsis grossulariae, *C. selachodon*	ITS 1,2	Identification, bioecology	Fenton et al. 1996
Cecidophyopsis grossulariae, *C. ribis*, *C. spicata*	ITS 1	Identification	Kumar et al. 1999
Cecidophyopsis alpina, *C. aurea*, *C. grossulariae*, *C. psilaspis*, *C. ribis*, *C. selachodon*, *C. spicata*	ITS 1,2	Phylogeny related to host association	Fenton et al. 2000
Cecidophyopsis alpina, *C. ribis*, *C. selachodon*, *C. spicata*	ITS 1	Identification	Lemmetty et al. 2001
Aceria cajani	ITS 1,2	Assess intraspecific variation	Kumar et al. 2001
Colomerus vitis, *Calepitrimerus vitis*	ITS 1, microsatellite	Identification, cryptic species	Carew et al. 2004
Aceria guerreronis	16S, ITS 1,2	Phylogeography, invasive routes	Navia et al. 2005
Floracarus perrepae	COI; D2	Assess intraspecific variation	Goolsby et al. 2006
Aceria tosichella	16S; ITS1; ANT	Identification, cryptic species	Carew et al. 2009
Acabarus hystrix	COI; D2	Identification, cryptic species	Skoracka and Dabert in press

species associated with *A. tosichella*, however limited sequence variation was observed (Carew et al. 2004). In addition, microsatellite loci have been used by Carew et al. (2004) to evaluate the population structure of a grapevine pest *Colomerus vitis* (Pagenstecher). Using these diverse DNA-based markers (see Table 2), several questions could be addressed on systematics, plant-mite interaction and colonisation patterns in bioinvasions of eriophyoid mites, as summarized below.

Species identification

Eriophyoidea systematic based exclusively on morphological characters may present several limitations. Because of the considerable reduction and simplification in the body plan of eriophyoids, the structures that can be used for eriophyoid morphologically-based systematics are scarce compared to most of the other mites. Another limitation of some species is the absence of ontogenetic diversity as well as the lack of useful characters specific to the adult male (Lindquist and Amrine 1996). An obvious significant cause of mistakes in eriophyoid systematics is the occurrence of deuterogyny (the life cycle usually including two forms of females and one form of male). The two forms of females—protogyne and deutogyne—might have been described as two different taxa often based on their morphological characters letting systematicists to classify them in different genera (Manson and Oldfield 1996). As a consequence, many species are probably junior synonyms of their correspondent deuytogyne/protogyne. It also would be necessary to

establish new combinations for species that were described based on deutogynes when its correspondent protogyne should be classified in a different genus. It is now widely accepted that molecular tools can help to clarify the systematics of taxa presenting such kind of misleading situations. Several examples combining data on nucleotide variation to more traditional morphological features help in establishing reliable criteria to determine species in the Eriophyoidea.

A series of studies on species identification, phylogeny and intraspecific variability in the *Cecidophyopsis* genus, have been conducted since 1995 (Fenton et al. 1995, 1996, 1997, 2000). This group includes mite species known to occur on twelve plant species of the genus *Ribes* and several of them are serious agricultural pests. Some species feed in young buds, causing galls resulting in the sterility of the flower buds and leading to considerable crop losses (De Lillo and Duso 1996). In addition, at least one species, *Cecidophyopsis ribis* (Westwood), transmits the agent of the reversion disease, the most important virus disease of blackcurrants, *Ribes nigrum* L., worldwide (Oldfield and Proeseler 1996). A PCR multiplex technique based on species-specific differences of the ITS-1 sequences, was developed to identify *Cecidophyopsis* mites (Kumar et al. 1999). The PCR multiplex technique presented in Kumar et al. (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—the bud mite and the blister mite, *C. vitis*, and the rust mite, *Calepitrimerus vitis* Nalepa—are well documented pests. The bud mite and blister mite while morphologically identical are thought to represent two strains of one species based on the type of damage they cause to grapes. The identity of these mites has recently been investigated using molecular markers (Carew et al. 2004). Two types of markers—PCR-RFLP of the ITS-1 and microsatellites—were used to gain insights into the biology and population structure. Results suggested that the bud and blister mite are different entities and should then be treated as separate agricultural problems with distinct control strategies. Importantly, infestations of vineyards with the blister mite will not result in outbreaks of the bud mite.

An important issue that molecular tools can help to address is testing hypotheses of the occurrence of cryptic species. The eriophyid *A. hystrix* has long been considered as a generalist species, having been reported from grasses of at least 30 genera, it was considered as unlikely evolving host specialization. Some studies however aimed to discriminate between three populations of *A. hystrix* from quackgrass, ryegrass and smooth brome. Using sequences of the mitochondrial COI gene and the nuclear D2 region of the 28S rDNA showed that populations from different grasses form distinct clades supporting the hypothesis of *A. hystrix* as being a complex of species and not a single generalist eriophyid taxon (Skoracka and Dabert in press). Likewise, several authors have suggested the occurrence of cryptic species within the wheat curl mite, *A. tosichella*, through morphological variation, although this could never be conclusively demonstrated. Using the mitochondrial 16S rRNA gene and two nuclear—ITS 1 and ANT—regions, it was shown that this taxon from Australia consists of at least two separate lineages that may represent putatively distinct species (Carew et al. 2009).

Plant-mite interaction and pest management

A valuable application of using genetic data to assist pest management is to investigate specialisation of mites to their host plant, which in some cases have uncovered host races. Although poorly used in eriophyoids, the approach has been used to investigate some

economically important issues. The eriophyid mite *Aceria cajani* (Channabasavanna) is the vector of the agent of pigeonpea sterility mosaic disease (PSMD), a very damaging virus-like disease in the Indian subcontinent (Ghanekar et al. 1992). When this disease occurs early in the season, yield losses can reach over 90%. 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 nucleotide sequences variation and patterns of restriction enzymes in the ITS region and associated rDNA genes (Kumar et al. 2001). Results strongly suggested that *A. cajani* on pigeonpea across the Indian subcontinent constitutes a single species. It could be concluded that no other *Aceria* species and probably no *A. cajani* biotypes that differ in their vectoring ability are involved in the transmission of the agent of PSMD. It seems most likely therefore that this variation in resistance is due to the occurrence of strains of the PSMD agent and host interaction with these strains.

The phylogenetic relationship of seven species of *Cecidophyopsis* mites—*C. ribis*, *C. selachodon* Eyndhoven, *C. spicata* Jones, *C. grossulariae* (Collinge), *C. alpine* Amrine, *C. aurea* Amrine and *C.* n.sp.—with its *Ribes* hosts—*R. nigrum* L., *R. sativum* (Reichb.), *R. petraeum* Wulfen, *R. rubrum* L., *R. spicatum* Robson, *R. grossularia* L., *R. oxyacanthoides* L., *R. alpinum* L. and *R. aureum* Pursh—was inferred from sequences of the ITS region (Fenton et al. 2000). In addition, a phylogenetic analysis of the associated host plants was conducted. The comparison of the two phylogenetic trees (mites versus hosts) clearly displayed divergent topologies, showing that the mite speciation did not closely follow host plant speciation events. Instead, the three groups of *Ribes*-infesting *Cecidophyopsis* mites derived from a common galling ancestor millions of years ago. Each mite group has recently diversified onto different primary hosts. One group of mites has even lost the galling ability. The results indicated that the speciation process in *Cecidophyopsis* mites has not followed that of their *Ribes* hosts, the later being a much more recent event. The results have implications for the host range and durability of mite-resistance in cultivated *Ribes*.

As part of a biological control program for *Lygodium microphyllum* (Cav.) R. Br., an invasive climbing fern in Florida, USA initiated in 1997, surveys for natural enemies were conducted in the native range of the plant, which includes Australia, Asia and Oceania. Among the herbivores documented the eriophyoid *F. perrepae* causes heavy plant damage and has a high impact on host biomass production. Several genotypes of *F. perrepae* from New Caledonia, China, Thailand, India/Sri Lanka and Cape York and Queensland in Australia, were identified based in mitochondrial (COI) and nuclear (D2 domain two gene region of the 28s rRNA gene) sequences. The different mite genotypes were tested for acceptance of the invasive Florida genotype of the climbing fern. Populations from Cape York performed best and were selected to be introduced in Florida (Goolsby et al. 2006).

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

The tremendous economic impact caused by invasive species has received increasing attention and motivated much research aimed at understanding invasive processes (Grbic et al. 2007; Facon et al. 2008) and help to establish control measures (Fagan et al. 2002). One valuable approach is the study of sources and introduction routes of invasive arthropods facilitated by the use of molecular markers (Darling and Blum 2007).

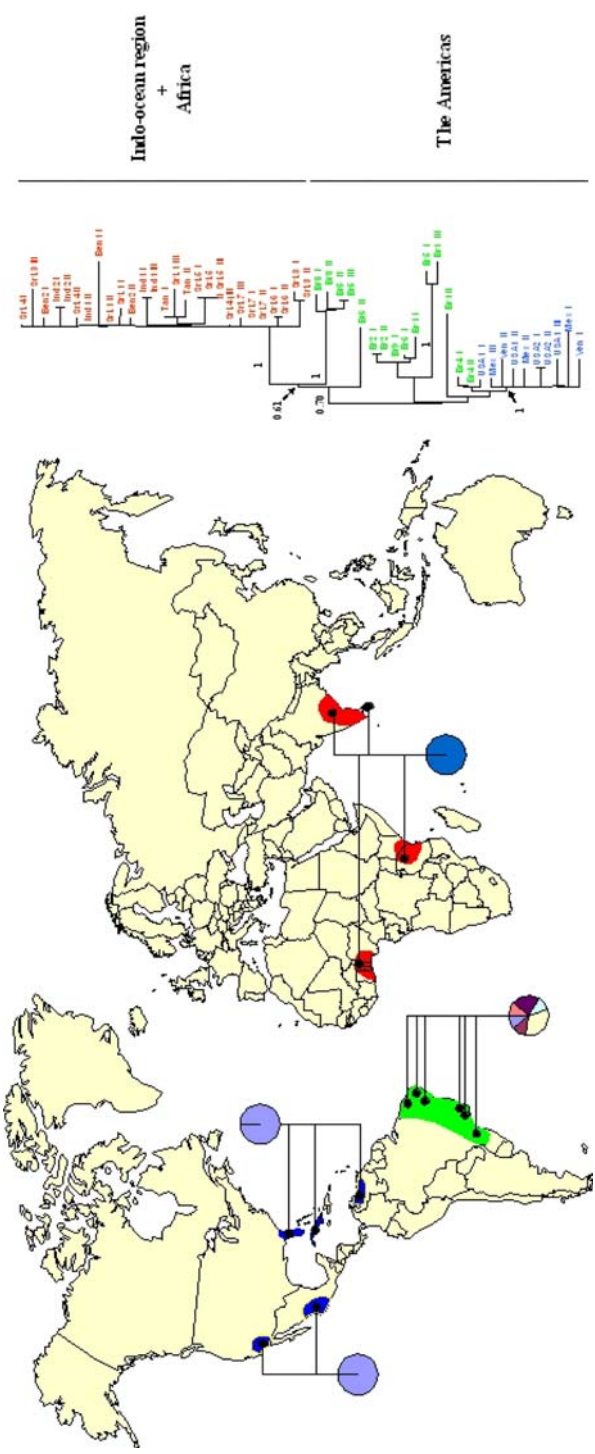

Fig. 2 Phylogeographical history of the coconut mite. The three geographical regions sampled are indicated in *red* (Indo-ocean region), *green* (Brazil) and *blue* (other American countries) **a** different mitochondrial haplotypes detected and their frequency in the different sampled localities (*black 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 (Color figure online)

Molecular information has been used to trace the invasion routes of plant mites, including the eriophyoid coconut mite, *A. guerreronis*. This species has recently spread and rapidly established in important coconut production areas worldwide, being considered as a highly destructive invasive species. The mite has not been recorded in the Indo-Pacific region, the area of origin of the coconut, suggesting that it has infested coconut only recently. In a recent phylogeography study conducted to investigate the geographical origin, ancestral host associations, and colonization history of the mite (Navia et al. 2005), DNA sequences variation from one mitochondrial (16S) and one nuclear region (ITS) were used to investigate mites obtained from samples of 29 populations originating from The Americas, Africa and the Indo-ocean region, the three continents where the mite has been reported (Fig. 2). Mitochondrial sequences were most diverse in Brazil (six out of a total of seven haplotypes recorded). A single haplotype was shared by non-American mites. Accordingly, patterns of nuclear ITS variation was similar, with the highest nucleotide diversity found in Brazil. The results suggested that the mite originates from The Americas and not from the ancestral region of coconut in South East Asia. Thus, colonization of coconut by this mite is a recent event, perhaps facilitated by modern transportation of coconut or propagation material potentially from a palm different from coconut. In addition, the fact that all samples from Africa and Asia were identical or very similar is consistent with the hypothesis that the mite invaded these regions recently and from a common source. The study prompts a reassessment of efforts using quarantine measures by knowing movements of the pest.

Critical technical aspects on the use of molecular methods to study eriophyoid biology

DNA extraction and voucher specimens

One feature that might have discouraged the development of molecular studies on eriophyoid mites is their reduced size. It can be noticed that in most studies on eriophyoid mites, authors have extracted DNA from a pool of specimens (5–20), especially for sequencing uses (e.g. Fenton et al. 2000; Kumar et al. 2001; Navia et al. 2005), except for Carew et al. (2004; 2009) that were successful in extracting DNA of individual eriophyid mites. This contrasts with the common use of single mites which has been reported for other groups of plant mites, e.g. Tetranychidae and Tenuipalpidae (Navajas et al. 2001; Rodrigues et al. 2004). However, the bias to analyze several mites as a single sample seems to be less important in eriophyoids considering that many species reproduce by arrenotokous parthenogenesis (Helle and Wysoki 1996). It is likely that specimens collected from one colony (gall, bud, blister, erineum species) are probably highly inbred and when pooling several specimens in a single sample the genetic diversity would be reduced. Caution however should be taken for species reproducing sexually, through transfer of spermatophore, and being vagrants. In this case, working with a pool of specimens can lead to a loss of information or even give misleading results because the analyzed sample might contain mites coming from distant areas and being genetically divergent. In this regard, it is worthy to notice that recent DNA extraction methods based on whole-genome pre-amplification have proved good results in terms of DNA yield from mites (Konakandla et al. 2006).

Most of the DNA extraction methods that have been used so far in studies with eriophyoid mites are destructive. Whole mites are crushed during the first steps of the extraction protocol, making impossible be used as a voucher specimen. Yet, all study on

DNA-based systematics should be associated to a voucher specimen whose origin and current status should be registered (Ruedas et al. 2000; Marrelli et al. 2006). This requirement is reinforced when observing the growing problem of taxonomic misidentification in public DNA databases which threatens the utility of the deposited sequences database reported by authors working with different groups of organisms (Sperling et al. 1994; Ruedas et al. 2000; Bridge et al. 2003; Vilgalys 2003). It would be interesting to use a nondestructive DNA extraction protocol which allows to preserve vouchers for morphological identification after DNA extraction (Rowley et al. 2007). An interesting DNA extraction protocol have been described and used for feather mites (Analgoidea) which makes possible to prepare slides of specimens after DNA extraction (Dabert et al. 2008). This same protocol was successfully used for *A. hystrix*; exoskeletons of the eriophyoid mites which had been stored in 70% ethyl alcohol until used for preparing microscopic slides (Skoracka and Dabert in press).

Collaboration between molecular and taxonomy experts

As for any taxa, the data on eriophyoid systematics obtained by DNA data would be a more efficient tool if combined with classical systematic information. In some cases, collaboration between both molecular and morphological expertise seems to lack. For example, the work by Carew et al. (2004) based on molecular data supported the occurrence of two taxa which before were considered as "strains" of *C. vitis* in Australia. While useful in defining taxonomical units, these studies would have beneficiated from a detailed morphological study aimed at detecting the characters that could discriminate taxa. Thus, molecular information while helping to detect and define the occurrence of cryptic species has not been concomitantly used by classical taxonomists to guide detailed morphological or morphometric studies in the search for diagnostic characters to be used in the description of new taxa.

Molecular techniques to study eriophyids: new avenues

DNA is progressively invading the field of Acarology. The minute eriophyoids is increasingly benefitting from the fast improvement of techniques to define and use molecular markers by adopting new forms and innovative approaches to detect DNA polymorphism. As a result, much can now be expected from the molecular techniques to gain in knowledge of the eriophyoid. Some of the main issues that we think should take advantage of the molecular approaches in the near future are discussed below.

Although the monophyly of the group is mostly accepted, its relationship to other Prostigmata remains to be investigated. The most recent phylogenetic hypothesis of the Eriophyoidea places the superfamily as a sister group of the Tydeoidea on the basis of a number of characters, none of which are synapomorphies (Lekveishvili et al. 2008). Likewise, the majority of taxonomic groups within the Eriophyoidea are artificial (Lindquist and Amrine 1996). As a result, the current classification has little predictive power. The lack of information on the Eriophyoidea phylogeny has been an important limitation on the progress of the systematics and biology of the group. As an example, the present classification does not reflect patterns of evolution and adaptation of these mites to their host plants (Lindquist and Amrine 1996), limiting the use of this knowledge to solve applied questions, as for instance in detecting host-adapted populations of crop pests. The reconstruction of Eriophyoidea phylogeny using molecular data remains a challenge,

which can nowadays be addressed thanks to methodological advances. For lower taxonomic levels, uncertainties on Eriophyoidea systematics are numerous and molecular techniques could help to answer questions and test hypothesis of different nature, e.g. synonymies and occurrence of cryptic species. For more applied issues, information provided through molecular studies can significantly contribute to define management strategies for eriophyoid pests. A significant pest control concern is the use of eriophyoid mites as biological control agents of weeds or other invasive plants. The knowledge of the genetic intraspecific variability of a biological control agent is the basis to establish efficient biological control strategies. The intraspecific variability is also relevant information on species that are vectors of plant pathogens especially when the pathosystem management should be based on the host genetic resistance. Diverse vector biotypes can present different levels of resistance to host plant genes involved in mite resistance. In addition, among the phytophagous mites, eriophyoids are being increasingly recognized for their potential to become invasive species (Hong et al. 2006). It is necessary to adopt preventive measures to avoid introductions and fast dissemination of eriophyoid pests in new geographical areas. Interception of potential invaders depends on a detailed inspection followed by a reliable and fast identification of the organisms. Information on routes of colonization or pathways of introduction is required to implement quarantine measures.

Looking to the future

Acarolgy is slowly entering the genomics era, with the whole genome sequencing projects of the tick *Ixodes scapularis* Say (Hill and Wikel 2005) and the spider mite *T. urticae* (Grbic et al. 2007) being completed and other sequencing projects that have already been proposed (see http://www.ncbi.nlm.nih.gov/sites/entrez). No doubt that eriophyoids will benefit from the new avenues permitted by these resources and more broadly Acarology will benefit from the technical advances made in other arthropods. With the advent of the genomic tools, that the whole genome projects will provide, the study of biological complex traits to address a diversity of problems caused by arthropod pest (Edwards 2009) in agriculture will be facilitated as reviewed in Heckel (2003) and Grimmelikhuijzen et al. (2007).

References

Amrine JW Jr, De Lillo E (2006) A database on Eriophyoidea of the world. West Virginia University

Behura SK (2006) Molecular marker systems in insects: current trends and future avenues. Mol Ecol 15:3087–3113

Ben-Ali Z, Boursot P, Said K, Lagnel J, Chatti N, Navajas M (2000) Comparaison of ribosomal ITS regions among *Androctonus* spp. scorpions (Scorpionida: Buthidae) from Tunisia. J Med Entomol 37:787–790

Ben-David T, Melamed S, Gerson U, Morin S (2007) ITS2 sequences as barcodes for identifying and analyzing spider mites (Acari: Tetranychidae). Exp Appl Acarol 41:169–181

Bridge PD, Spooner BM, Panchal G (2003) On the unreliability of published DNA sequences. New Phytol 160:43–48

Carbonnelle S, Hance T, Migeon A, Baret PV, Cros-Arteil S, Navajas M (2007) Microsatellite markers reveal spatial genetic structure of *Tetranychus urticae* (Acari: Tetranychidae) populations along a latitudinal gradient in Europe. Exp Appl Acarol 41:225–241

Carew ME, Goodisman MAD, Hoffmann AA (2004) Species status and population genetic structure of grapevine eriophyoid mites. Entomol Exp Appl 111:87–96

Carew M, Schiffer M, Umina P, Weeks A, Hoffmann A (2009) Molecular markers indicate that the wheat curl mite, *Aceria tosichella* Keifer, may represent a species complex in Australia. Bull Entomol Res 99:479–486

Caterino MS, Cho S, Sperling FAH (2000) The current state of insect molecular systematics: a thriving tower of Babel. Ann Rev Entomol 45:1–54
Cusson M (2008) The molecular biology toolbox and its use in basic and applied insect science. Bioscience 58:691–700
Dabert J, Ehrnsberger R, Dabert M (2008) *Glaucalges tytonis* sp.n. (Analgoidea, Xolalgidae) from the barn owl *Tyto alba* (Strigiformes, Tytonidae): compiling morphology with DNA barcode data for taxon descriptions in mites (Acari). Zootaxa 1719:11
Darling JA, Blum MJ (2007) DNA-based methods for monitoring invasive species: a review and prospectus. Biol Invasions 9:751–765
De Lillo E, Duso C (1996) Currants and berries. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites—their biology, natural enemies and control. Elsevier, Amsterdam, pp 583–591
DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philos Trans R Soc Lond B Biol Sci 360:1905–1916
DeYoung RW, Honeycutt RL (2005) The molecular toolbox: genetic techniques in wildlife ecology and management. J Wildl Manage 69:1362–1384
Edwards S (2009) Is a new and general theory of molecular systematics emergins? Evolution 63:1–19
Excoffier L, Heckel G (2006) Computer programs for population genetics data analysis: a survival guide. Nat Rev Genet 7:745–758
Facon B, Pointier JP, Jarne P, Sarda V, David P (2008) High genetic variance in life-history strategies within invasive populations by way of multiple introductions. Curr Biol 18:363–367
Fagan W, Lewis M, Neubert M, van den Driessche P (2002) Invasion theory and biological control. Ecol Lett 5:148–157
Fenton B, Malloch G, Jones AT, Amrine JW Jr, Gordon SC, A′Hara S, McGavin WJ, Birch ANE (1995) Species identification of *Cecidophyopsis* mites (Acari: Eriophyidae) from different *Ribes* species and countries using molecular genetics. Mol Ecol 4:383–387
Fenton B, Jones AT, Malloch G, Thomas WP (1996) Molecular ecology of some *Cecidophyopsis* mites (Acari: Eriophyidae) on *Ribes* species and evidence for their natural cross colonisation of blackcurrant (*R. nigrum*). Ann Appl Biol 128:405–414
Fenton B, Malloch G, Moxey E (1997) Analysis of eriophyid rDNA internal transcribed spacer sequences reveals variable simple sequence repeats. Insect Mol Biol 6:23–32
Fenton B, Birch ANE, Malloch G, Lanham PG, Brennan RM (2000) Gall mite molecular phylogeny and its relationship to the evolution of plant host specificity. Exp Appl Acarol 24:831–841
Galtier N, Daubin V (2008) Dealing with incongruence in phylogenomic analyses. Philos Trans R Soc Lond B Biol Sci 363:4023–4029
Garant D, Kruuk L (2005) How to use molecular marker data to measure evolutionary parameters in wild populations. Mol Ecol 14:1843–1859
Gariepy TD, Kuhlmann U, Gillott C, Erlandson M (2007) Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of Arthropods. J Appl Entomol 131:225–240
Ghanekar AM, Sheila VK, Benival SPS, Reddy MV, Nene YL (1992) Sterility mosaic of pigeonpea. In: Singh US, Mukhopadhyay AN, Kumar J, Chaube HS (eds) Plant diseases of international importance, vol 1. Prentice Hall, New Jersey, pp 415–428
Goldstein DB, Schlötterer C (1999) Microsatellites, evolution and applications. Oxford University Press, New York, p 352
Goolsby JA, De Barro PJ, Makinson JR, Pemberton RW, Hartley DM, Frohlich DR (2006) Matching the origin of an invasive weed for selection of a herbivore haplotype for biological control programme. Mol Ecol 15:287–297
Grbic M, Khila A, Lee K-Z, Bjelica A, Grbic V, Whistlecraft J, Verdon L, Navajas M, Nagy L (2007) Mite model: *Tetranychus urticae*, a candidate for chelicerate model organism. Bioessays 29:489–496
Grimmelikhuijzen CJP, Cazzamali G, Williamson M, Hauser F (2007) The promise of insect genomics. Pest Manag Sci 63:413–416
Heckel DG (2003) Genomics in pure and applied Entomology. Ann Rev Entomol 48:235–260
Helle W, Wysoki M (1996) Arhenotokous parthenogenesis. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites: their biology, natural enemies and control. Elsevier, Amsterdam, pp 169–171
Hill CA, Wikel SK (2005) The *Ixodes scapularis* Genome Project: an opportunity for advancing tick research. Trends Parasitol 21:151–153
Hinomoto N, Tran DP, Pham AT, Le TBN, Tajima R, Ohashi K, Osakabe M, Takafuji A (2007) Identification of spider mites (Acari: Tetranychidae) by DNA sequences: a case study in Northern Vietnam. Int J Acarol 33:53–60
Hong XY, Wang DS, Zhang ZQ (2006) Distribution and damage of recent invasive eriophyoid mites (Acari: Eriophyoidea) in mainland China. Int J Acarol 32:227–240

Konakandla B, Park Y, Margolies D (2006) Whole genome amplification of Chelex-extracted DNA from a single mite: a method for studying genetics of the predatory mite *Phytoseiulus persimilis*. Exp Appl Acarol 40:241–247

Kumar L, Fenton B, Jones AT (1999) Identification of *Cecidophyopsis* mites (Acari: Eriophyidae) based on variable simple sequence repeats of ribosomal DNA internal transcribed spacer-1 sequences via multiplex PCR. Insect Mol Biol 8:347–357

Kumar PL, Fenton B, Duncan GH, Jones AT, Sreenivasulu P, Reddy DVR (2001) Assessment of variation in *Aceria cajani* using analysis of rDNA ITS regions and scanning electron microscopy: implications for the variability observed in host plant resistance to pigeonpea sterility mosaic disease. Ann Appl Biol 139:61–73

Lekveishvili M, Amrine JW Jr, Wells J (2008) Preliminary results of eriophyoid phylogeny (Acari: Prostigmata). In Sixth European Congress of Acarology. European Association of Acarologists, Montpellier, 49

Lemmetty A, Tikkanen M, Tuovinen T, Lehto K (2001) Identification of different *Cecidophyopsis* mites on Ribes in Finland. Acta Hortic 656:115–118

Lindquist EE, Amrine JWJ (1996) Systematics, diagnoses for major taxa, and keys to families and genera with species on plants of economic importance. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites: their biology, natural enemies and control. Elsevier, Amsterdam, pp 33–88

Manson DCM, Oldfield GN (1996) Life forms, deuterogyny, diapause and seasonal development. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites: their biology, natural enemies and control. Elsevier, Amsterdam, pp 173–183

Marrelli MT, Sallum MAM, Marinotti O (2006) The second internal transcribed spacer of nuclear ribosomal DNA as a tool for Latin American anopheline taxonomy—a critical review. Mem Inst Oswaldo Cruz 101:817–832

Mullis KB, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Meth Enzymol 155:335–350

Navajas M, Boursot P (2003) Nuclear ribosomal DNA monophyly *versus* mitochondrial DNA polyphyly in two closely related mite species: the influence of life history and molecular drive. Proceedings of the Royal Society of London B (Suppl) 270: S124–S127

Navajas M, Fenton B (2000) The application of molecular markers in the study of diversity in acarology: a review. Exp Appl Acarol 24:751–774

Navajas M, Gutierrez J, Williams M, Gotoh T (2001) Synonymy between two spider mite species, *Tetranychus kanzawai* and *T. hydrangeae* (Acari: Tetranychidae), shown by ribosomal ITS2 sequences and cross-breeding experiments. Bull Entomol Res 91:7

Navia D, GJd Moraes, Roderick G, Navajas M (2005) The invasive coconut mite *Aceria guerreronis* (Acari: Eriophyidae): origin and invasion sources inferred from mitochondrial (16S) and nuclear (ITS) sequences. Bull Entomol Res 95:505–516

Nichols R (2001) Gene trees and species trees are not the same. Trends Ecol Evol 16:358–364

Oldfield GN, Proeseler G (1996) Eriophyoid mites as vectors of plant pathogens. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites—their biology, natural enemies and control. Elsevier, Amsterdam, pp 259–275

Rodrigo A, Bertels F, Heled J, Noder R, Shearman H, Tsai P (2008) The perils of plenty: what are we going to do with all these genes? Philos Trans R Soc Lond B Biol Sci 363:3893–3902

Rodrigues JCVR, Childers CC, Gallo-Meagher M, Ochoa R, Adams BJ (2004) Mitochondrial DNA and RAPD polymorphisms in the haploid mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae). Exp Appl Acarol 34:17

Rowley DL, Coddington JA, Gates MW, Norrbom AL, Ochoa RA, Vandenberg NJ, Greenstone MH (2007) Vouchering DNA-barcoded specimens: test of a nondestructive extraction protocol for terrestrial arthropods. Mol Ecol Notes 7:915–924

Ruedas LA, Salazar-Bravo J, Dragoo JW, Yates TL (2000) The importance of being earnest: what, if anything, constitutes a "specimen examined?". Mol Phylogenet Evol 17:129–132

Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding. Philos Trans R Soc Lond B Biol Sci 360:1805–1811

Shendure J, Ji HL (2008) Next-generation DNA sequencing. Nat Biotechnol 26:1135–1145

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701

Skoracka A, Dabert M (in press) The cereal rust mite *Abacarus hystrix* (Acari Eriophyoide) is a complex of species: evidences from mitochondrial and nuclear DNA sequences. Bull Entomol Res. doi: 10.1017/S0007485309990216

Sonnenberg R, Nolte AW, Tautz D (2007) An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. Front Zool 4:12
Sperling F, Anderson G, Hickey D (1994) A DNA-based approach to the identification of insect species used for postmortem interval estimation. J Forensic Sci 39:418–427
Sunnucks P (2000) Efficient genetic markers for population biology. Trends Ecol Evol 15:199–203
Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP (2003) A plea for DNA taxonomy. Trends Ecol Evol 18:70–74
Vilgalys R (2003) Taxonomic misidentification in public DNA databases. New Phytol 160:3–4