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Molecular variation of the cytochrome b DNA and protein sequences in *Phytoseiulus macropilis* and *P. persimilis* (Acari: Phytoseiidae) reflect population differentiation

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Abstract
Several phytoseiid mite species are important natural enemies used in biological control strategies. In the present study, Cytb mtDNA sequences of various populations of two species, *Phytoseiulus macropolis* and *P. persimilis*, were compared to determine whether the specimens collected in Brazil could belong to *P. persimilis* as this latter species is reported in South America but not in Brazil. The Cytb marker was used because of its high evolution rate, assumed to capture intraspecific variation. No overlap between intra- and interspecific distances was observed but the distances were quite low for interspecific variation. This can be due to the particular biology of *Phytoseiulus* species and this shows the difficulty to apply a universal threshold in genetic distances to conclude about the existence of one or several species. Cytb mtDNA sequences were also considered to assess intraspecific variation. The DNA sequences of *P. persimilis* populations were very similar, probably because they all originated from the West Palearctic region or because of a prevalence of commercialized specimens *in natura*. For *P. macropilis*, higher genetic distances were observed and differentiation was noted according to geographic location and, to a smaller extent, pyrethroid resistance. To determine how DNA variation might impact the protein function (CytB fragment considered), the amino acid compositions of the populations studied were compared. No diagnostic mutation was observed between pyrethroid resistant and susceptible populations, whereas four mutations were identified between populations of *P. macropilis* separated by 1300 km (different climatic conditions). The impact of such mutations is discussed but knowledge is scarce, which makes it difficult to root testable hypotheses. The protein analysis clearly opens new perspectives in Phytoseiidae studies.

Keywords Resistance · Cytochrome B mtDNA · Protein structure · Phytoseiidae · Taxonomy

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Introduction

Many Phytoseiidae mites are efficient predators of pest mites and little insects (Gerson et al. 2003). Some species are commercialized all over the world for biological control issues. Among these species, two belong to the genus *Phytoseiulus*: *Phytoseiulus persimilis* Athias-Henriot and *Phytoseiulus macropilis* Banks. Both species are specialist predators (McMurtry et al. 2013). They preferentially prey on *Tetranychus urticae* Koch (Acari: Tetranychidae), a polyphagous and cosmopolitan species, feeding on—and damaging—more than 1100 plant species (140 families) (Takafuji 1977; Zhang 2003; Escudero and Ferragut 2005; Grbić et al. 2011). *Phytoseiulus persimilis* is the oldest commercialized predatory mite and is more widely spread than *P. macropilis* (Demite et al. 2020). This latter species, also commercialized, is reported in various continents, but is most frequently encountered in South America, the supposed origin centre of the genus *Phytoseiulus* (Kanouh et al. 2010; Demite et al. 2020). *Phytoseiulus macropolis* and *P. persimilis* are morphologically very close, only differing by the number of setae on the ventrianal shield (McMurtry 1983; Takahashi and Chant 1993a; Kanouh et al. 2010). Several studies—including morphological and molecular approaches, and crosses—seem to show that they are distinct species (Takahashi and Chant 1993b; Okassa et al. 2010). However, one question remains about the identity of these two species in Brazil.

Phytoseiulus persimilis is reported all over the world, including South America, but not in Brazil, whereas *P. macropilis* is present in Brazil. The absence of *P. persimilis* in Brazil thus seems strange and we can wonder whether some of the specimens identified as *P. macropilis* in Brazil actually belong to *P. persimilis*. The present study aims to provide additional elements to the study of Okassa et al. (2010), including many more specimens of both species. The second objective of this study is to assess genetic variation at the intraspecific level. Genetic variation within a species can be affected by several factors, such as host plants (as shown for *Phytoseius finitimus* Ribaga; Tixier et al. 2017), prey (as shown for *Phytoseiulus longipes* Evans; Tixier et al. 2010a), and geographical distribution (as shown for *Amblyseius largoensis* (Muma); Bowman and Hoy 2012; Navia et al. 2014; Barbosa Lima et al. 2018). Here, we investigate how molecular genotype reflects the differentiation of the populations according to geographical distances and resistance to pesticides, as one of the *P. macropilis* populations was resistant to pyrethroids.

There is a great challenge in early characterization of resistance in field populations for determining resistance persistence and dissemination. For this, we choose to use a neutral marker, the cytochrome b mtDNA (Cytb) fragment. Mutation in this DNA fragment has been associated with resistance to bifenazate, acequinocyl and other mitochondrial Qo inhibitors, in Tetranychidae mites (Van Leeuwen et al. 2008, 2011; Van Nieuwenhuyse et al. 2009; Fotoukkiaii et al. 2020). In the present case, a population is resistant to pyrethroids and the resistance mechanisms are obviously different to Qo inhibitor acaricide resistance. The Cytb marker was considered as neutral for this strait, and was chosen because of its high evolution rate and great intraspecific variation within Phytoseiidae species (Dos Santos and Tixier 2017; Tixier et al. 2017, 2019). We thus assumed that this marker would be appropriate to capture intraspecific variation.

Material and methods

Species and populations studied

The characteristics of the species and populations considered are described in Table 1. Eight populations of *P. macropilis* collected in two states of Brazil (Sao Paulo and Rio Grande do Sul) and in Argentina were considered. Some populations were collected in open-field crops and greenhouses (population A was collected on gerbera, I and L on strawberry, and V on *Sechium elude*). One population is a commercialized population (P, obtained from Promip, Limeira, SP, Brazil). Population R, initially collected in 2010 from a strawberry field in State of São Paulo, is maintained in the Acarology Laboratory of the Instituto Biológico (Campinas, SP). This population is known for its resistance to fenpropathrin (Queiroz and Sato 2016) and has been kept in the laboratory under selection pressure. It is now approximately 5300×, 738×and 735×more resistant to fenpropathrin, bifenthrin and deltamethrin than a susceptible population, respectively (Queiroz and Sato 2016). Population C has colonized the rearing units of *T. urticae* in the Acarology Laboratory of Instituto Biológico and its origin is unknown. Four DNA sequences (deposited in GenBank) of a population of *P. macropilis* collected in Argentina were also considered (Okassa et al. 2010).

The 26 specimens of *P. persimilis* considered were collected in the South of Europe and Northern Africa (France, Spain, Tunisia and Italy) on crops and weeds. One population is a commercial one. Twenty-two sequences were those used in Okassa et al. (2010) and four were newly obtained.

Molecular analysis

DNA extraction

DNA extraction was carried out on a single female as described by Kanouh et al. (2010) using a DNeasy tissue kit (Qiagen, Hilden, Germany) adapted for total DNA extracting of mites. Mites were retrieved after DNA extraction and mounted on slides as described by Tixier et al. (2010b).

DNA amplification and sequencing

Cytochrome b mtDNA was chosen as this DNA fragment allows assessing recent evolutionary history and is sensitive to species and population differentiation (i.e., Dos Santos and Tixier 2017).

Primers and thermal cycling were those described in Tixier et al. (2012). The PCR reactions were performed in a volume of 25 µL, containing 4 µL of mite DNA, 2.5 µL (1 mM) of 10×buffer, 0.5 µL (25 mM) of $MgCl_2$, 0.5 µL (2.5 mM) DNTPs, 0.175 µL (10 µmM) of each primer, 0.5 (10 mg/mL) BSA, 0.125 µL (5 U) of Taq Qiagen and 13.375 µL of water. Electrophoresis was performed on 1.5% agarose gel in 0.5×TBE buffer for 30 min at 100 V. PCR products were sequenced using the dynamic ET terminator cycle sequencing kit. DNA Purification was carried out with Exosap-IT (Amersham). The sequencer used was the Megabase 1000 apparatus. All DNA fragments were sequenced along both strands. A preliminary analysis was conducted to check for the

Table 1 Characteristics of the various populations of *Phytoseiulus macropilis* and *P. persimilis* considered, with plant support species and accession numbers in the GenBank database (in bold, the accession numbers of the sequences obtained in the present study)

Species	Population code	Plant	Locality and collection date	GPS coordinates	No. specimens sequenced	GenBank accession numbers
P. macropilis	A	*Gerbera* sp.	Holambra, São Paulo (SP), Brazil, 2019 Collected in a 180 m^2 greenhouse	22°54′54.54″S, 47°02′12.57″W	8	**MT110237–MT110244**
	C	Laboratory contaminations in *Tetranychus urticae* rearings	Campinas, SP, Brazil, 2019	22°54′12.19″S, 47°00′53.50″W	3	**MT110245–MT110247**
	I	*Fragaria* sp. (in greenhouse)	Pinhalzinho, SP, Brazil, 2019 Collected in a 350 m^2 greenhouse	22°46′51.04″S, 46°35′26.76″W	10	**MT110248–MT110257**
	L	Reared in the laboratory since 2019 and initially collected on *Fragaria* sp.	Lageado, Rio Grande do Sul (RS), Brazil, 2019	29°26′40.98″S, 51°57′24.03″W	10	**MT110258–MT110267**
	P	Mass reared population – origin unknown	Commercial population (Promip), 2019	22°29′20.54″S, 47°11′49.95″W	10	**MT110268–MT110277**
	R	Population resistant to fenpropatrin (pyrethroids), and maintained under selection pressure for 10 years, initially collected on *Fragaria* sp.	Socorro, São Paulo (SP), Brazil, 2010	22°35′52.12″S, 46°28′0.86″W	10	**MT110287–MT110296**
	V	*Sechium elude*	Amparo, SP, Brazil, 2019 Collected in a field of 20,000 m^2	22°44′50.05″S, 46°43′30.42″W	9	**MT110278–MT110286**
		Solanaceae	Argentina	31°32′46.8″S, 60°45′42.48″W	4	FJ985091–FJ985094

Table 1 (continued)

Species	Population code	Plant	Locality and collection date	GPS coordinates	No. specimens sequenced	GenBank accession numbers
P. persimilis		Mass-reared	Rearing units	Not provided in Okassa et al. (2010)	3	FJ985083–FJ985085
		Unknown	Spain	Not provided in Okassa et al. (2010)	5	FJ985076–FJ985080
		Phaseolus vulgaris	France	Not provided in Okassa et al. (2010)	7	FJ985069–FJ985075
		Broussonetia papyrifera	France, Cabannes de Salaison	43°35′N, 4°00′E	1	**MT110297**
		Unknown	Sicily, Italy	Not provided in Okassa et al. (2010)	3	GU232491–GU232493
		Unknown	Tunisia	Not provided in Okassa et al. (2010)	4	GU232494–GU232497
		Solanum nigrum	Cap Bon, Tunisia	37°4′60.00″N, 11°2′20.00″E	1	**MT110298**
		Amaranthus reflexus	Cap Bon, Tunisia	37°4′60.00″N, 11°2′20.00″E	2	**MT110299–MT110300**

absence of stop codons. The sequences obtained were compared to those included in the GenBank database to identify possible contaminations. The sequences were aligned and analysed within MEGA v.6.0.6 (Tamura et al. 2013). Genetic distances (using the Kimura 2 parameter) were calculated to compare DNA sequences. This genetic distance is the most used for molecular species identification and its use will permit to compare the present results with those of previous studies. A maximum likelihood tree was constructed; the best-fit-substitution model (TrN + G) was determined by Modeltest v.3.07 (Posada and Crandall 1998) in (PAUP*, v.4.0b.10; Swofford 2002) through hierarchical likelihood-ratio tests. *Neoseiulus californicus* (McGregor) was used as an outgroup (GenBank accession number JF279241).

Amino acid sequences of the Cytb protein were also studied. The amino acid sequences of the partial Cytb fragment herein considered were obtained using http://insilico.ehu.es/translate/. The total amino acid sequences of two *T. urticae* specimens retrieved from GenBank (YP_001795379, ACI30848) were aligned with those of one specimen of *P. persimilis* and all the specimens of *P. macropilis* (using MEGA). Then mutations in amino acid associated to pesticide resistance in *T. urticae* were searched. Because we found mutations in amino acid sequences between two clades of *P. macropilis*, we wanted to determine the impact of those mutations on the protein structure and function. For this, we used Swiss-model (https://swissmodel.expasy.org/) and DynaMut (http://biosig.unimelb.edu.au/dynamut/) online tools (Rodrigues et al. 2018). The structure of the protein was obtained as well as the location of the mutations. The ΔΔG index (Gibbs free energy, i.e., the difference in folding free energy between wild type and mutant) was used to assess the impact of mutations on protein stability and dynamics.

Results and discussion

Molecular results are based on the alignment of 443 bp for the Cytb fragment considered.

Identity of the specimens of *Phytoseiulus macropilis* from Brazil

The specimens of *P. macropilis* and *P. persimilis* are included in two different well-sustained clades (Fig. 1). As clades could correspond to populations or species (both monophyletic groups) (Moritz and Cicero 2004; Collins and Cruickshank 2013), we also analysed the genetic distances within and between these phylogenetic groups. The genetic distances between the 64 DNA sequences of *P. macropilis* specimens ranged from 0 to 9.5% (mean ± SE = 3.3 ± 0.6%). The genetic distances between the 26 DNA sequences of the *P. persimilis* specimens ranged from 0 to 1.1% (0.1 ± 0.07%). These values clearly correspond to the intraspecific variation range already observed for this DNA fragment within the family Phytoseiidae (Dos Santos & Tixier 2017; Tixier et al. 2017). The specimens of *P. macropilis* collected in Brazil thus belong to the same species as the ones collected in Argentina.

The genetic distances between the DNA sequences of *P. persimilis* and *P. macropilis* range from 16.5 to 19.9% (mean ± SE = 17.7 ± 3.06%) and no overlap between genetic distances of *P. macropilis* and *P. persimilis* specimens is observed. According to the 'barcoding gap' hypothesis (Hebert et al. 2003), it seems thus that all the *P. macropilis* specimens herein considered do not belong to *P. persimilis*. An intraspecific genetic distance higher than 19% has been reported in *P. finitimus* (23%) and *Typhlodromus* (*Anthoseius*)

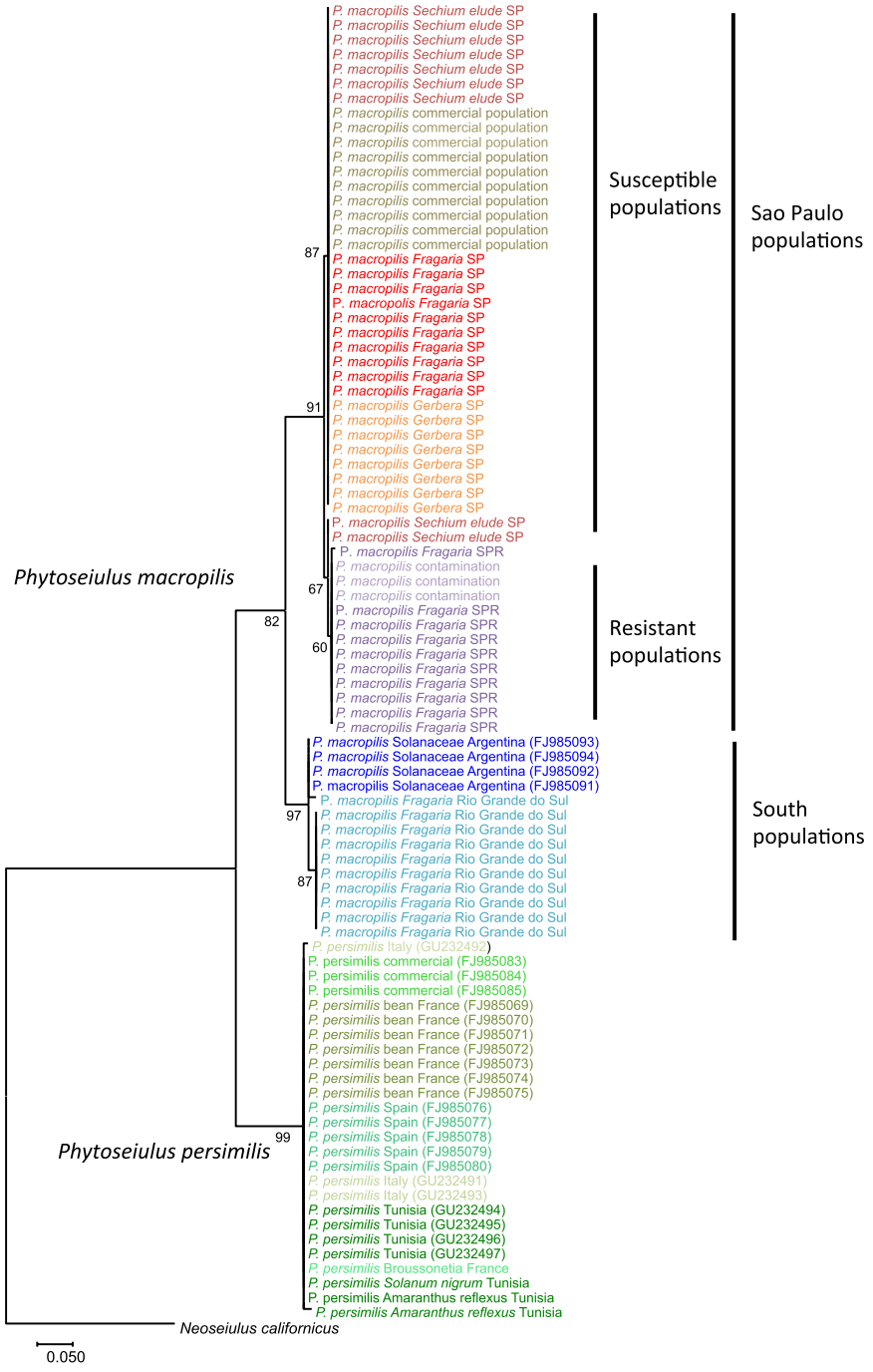

Fig. 1 Neighbour joining trees based on K2P genetic distances between the specimens of *Phytoseiulus persimilis* and *P. macropilis* with the Cytb mtDNA. The numbers at nodes correspond to bootstrap values

rhenanoides (Athias-Henriot) (21.7%) (Tixier et al. 2017, 2019). In both cases, these high intraspecific distances only concerned some specimens, whereas in the present case the high genetic distances between *P. macropilis* and *P. persimilis* concern all the specimens considered. Furthermore, the comparison with the interspecific distances obtained until now is quite difficult, as these distances are usually higher than 22%, concern morphologically different species, and many species of another Phytoseiidae subfamily (Typhlodrominae)—*P. macropilis* and *P. persimilis* belong to the subfamily Amblyseiinae (Tixier et al. 2019). Within the Amblyseinae, a genetic distance of 20% has been reported between *Neoseiulus idaeus* and *N. californicus* (Tixier et al. 2014). Okassa et al. (2010) found the distance between *P. persimilis* and *P. macropilis* to be about 14%, lower than the distance obtained in the current study—this is likely due to the much higher number of specimens presently considered.

As already stated for other arthropod groups, it seems difficult to apply a 'same decision threshold' based on intra- and interspecific overlap for all species of a single family because of very different biological features, evolution rates and speciation events (e.g., Hajibabaei et al. 2006; Van Velzen et al. 2012; Chapple and Ritchie 2013). The genus *Phytoseiulus* constitutes a quite particular Phytoseiidae group because of prey specificity, high reproductive parameters (rapid development cycle, high fecundity) and high dispersal ability among prey patches (McMurtry and Croft 1997). These biological traits could be associated to a higher gene flow than in other Phytoseiidae groups, a more recent speciation and, thus, to lower interspecific distances between two sister species (Avise and Ball 1990; Papadopoulou et al. 2008).

Finally, observations of the morphology of the specimens used for the molecular analysis showed that all the specimens of *P. macropilis* had the setae *JV2* on the ventrianal shield whereas the *P. persimilis* specimens had not, also suggesting that they are different species. The conclusion is the same as in Okassa et al. (2010) and Takahashi and Chant (1993b), who showed complete reproductive incompatibility between *P. persimilis* and *P. macropilis*. We thus conclude that *P. macropilis* and *P. persimilis* are two different species and the presently analysed *P. macropilis* specimens from Brazil do not belong to the species *P. persimilis*.

Genetic variation between the populations of *Phytoseiulus persimilis* and *P. macropilis*

The genetic variation between the DNA sequences of *P. persimilis* is very low. All specimens considered are similar to each other, including the commercialised ones. Only one specimen (from Tunisia) is a bit different (distance of 1.1%). It can thus be hypothesized (i) that the specimens collected might be issued from commercial releases, that spread in the environment (maybe associated to a sampling bias in places where this species has been frequently released), or (ii) that very little variation exists within the species *P. persimilis* in the considered area. However, because of our previous statement hypothesizing higher evolution rate in species of the genus *Phytoseiulus* (because of particular biological features), a high genetic diversity between populations was expected. For this reason, our first hypothesis seems the most probable, emphasizing the difficulty to find 'natural' specimens for species massively released for commercial issues. Finally, the low genetic distance might also show that the West Palearctic area—despite being the area where *P. persimilis* was first described by Athias-Henriot in 1957 (in Algeria)—is not the centre of origin of

this species. To test this hypothesis, it would be interesting to consider DNA sequences of *P. persimilis* from South America.

The genetic variation between the DNA sequences of *P. macropilis* is much higher than that observed for *P. persimilis*, even if all populations were collected in Brazil and sometimes in the same state (in very close areas). Globally, the intra-population distances are very low with a mean ranging from 0 to 0.42%, whereas the inter-population distances are much higher (see below). The phylogenetic tree shows two distinct clades (Fig. 1). Clade 1 contains specimens collected in Argentina on Solanaceae and specimens of the population 'L' collected in Rio Grande do Sul on strawberry (Rosaceae). Clade 2 contains the remaining populations all collected in the Sao Paulo state on different plants. The mean genetic distance between these two clades is 8.4% (range: 7.7–9.5%), the within-clade genetic distance mean being 0.5% (0–1.1%) for clade 1 and 0.63% (0–2.1%) for clade 2. No overlap between intra and inter-clade distances is observed, but the inter-clade distances clearly correspond to intraspecific variation (compared to previous references for Phytoseiidae mites), leading to the conclusion that these two clades belong to the same species even if no overlap in genetic distance is observed between intra and inter-population distances (Tixier et al. 2017, 2019).

This genetic differentiation could be associated to geographic location as populations of clade 1—although separated by 570 km—are geographically less distant to each other than to the populations of clade 2—all collected in Sao Paulo state (1300 km remote from

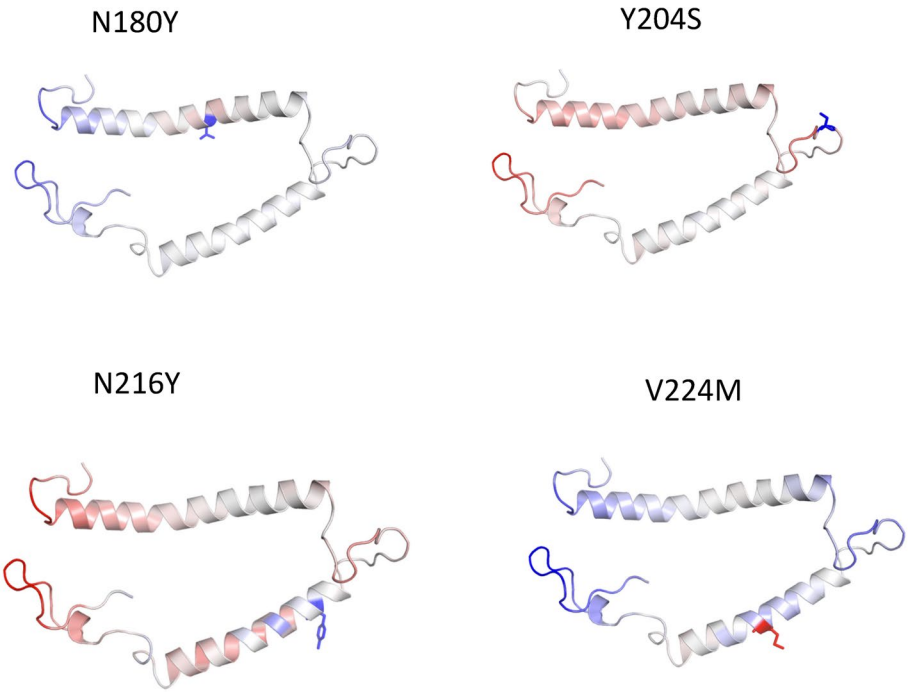

Fig. 2 Visual representation of vibrational entropy energy due to mutations N180Y, Y204S, N216Y and V224M in the Cytb protein of *Phytoseiulus macropilis*. Amino acids are coloured according to the vibrational entropy change upon mutation—blue represents a rigidification of the structure, red a gain in flexibility. (Color figure online)

specimens of clade 1). A differentiation within these two clades is also observed. In clade 1, specimens of the two populations ('L' and Argentina) are separated in two groups (by a weak mean genetic distance of 1.04%); this could again be explained by a geographical effect and/or by rearing pressure effects. *Phytoseiulus macropilis*, like all the species of the genus *Phytoseiulus*, has a high fecundity and a relatively high dispersion capacity especially to colonise new prey patches (Rosenheim et al. 2004). These biological features could explain founder effects and differentiation of populations in remote areas.

In clade 2, populations collected in the field and assumed to be susceptible to pesticides are included in a separate clade to the specimens resistant to pyrethroids ('R') and to the specimens of population 'C' whose origin was unknown. Because of the similarity between specimens 'R' and 'C', it seems that population 'C' is issued from some specimens of population 'R' that contaminated the *T. urticae* rearings. Two specimens among the nine of population 'V' are included in the 'resistant' clade even if a bit distant from the other specimens of this group. Population 'V' was collected in an area close to the initial collection locality of population 'R'. These two specimens could belong to the same population 'R' collected 10 years ago.

The bootstrap supporting this clade differentiation is not high; the mean genetic distance between resistant and susceptible groups is 1.12% and the within-population mean distances are 0% for the field populations and 0.06% for the resistant populations. This weak structuring reflects resistant *vs.* susceptible phenotypes, but with no functional association. Mutations have been reported on the Cytb amino acid sequence to explain resistance to bifenazate, acequinocyl and Qo inhibitors for *T. urticae* and *Panonychus ulmi* Koch (G132A, G126S, A133T, P262T, I136T, S141T, I260V, N326S; Van Leeuwen et al. 2008, 2011; Van Nieuwenhuyse et al. 2009; Fotoukkiaii et al. 2020). All specimens of *P. macropilis* have a wild-type amino acid sequence (a 'G' in position 132, an 'A' in position 133, a 'P' in position 262, an 'I' in position 136, an 'S' in position 141 and an 'I' in position 260). As expected, this shows that the specimens of *P. macropilis* are not resistant to bifenazate, acequinocyl and other Qo inhibitors which is in line with laboratory trials only showing resistance of the population 'R' to pyrethroids (Queiroz and Sato 2016). No diagnostic mutation was observed between 'susceptible' and 'resistant' populations.

Finally, one should be careful in associating the clades observed in resistant phenotypes, as the Cytb is a neutral marker for the pyrethroid resistance trait (no functional relationship between pyrethroid resistance and Cytb mutations); other selection pressures could explain the population differentiation within clade 2 (rearing pressure, biological features). The 'R' population has been reared—and insecticide resistance was selected for—for 10 years in the laboratory, so founder effects, genetic drift events and/or inbreeding could have lead to this particular differentiation from these other populations of *P. macropilis* from Sao Paulo state (e.g., Harshman and Hoffmann 2000; Roderick and Navajas 2003). DNA variation does not lead to amino acid change, showing rather synonymous mutations, not affecting the protein structure and function. Furthermore, no differentiation of field populations from the commercial population was observed, questioning the origin of these field populations (natural or released), with a low diversity potentially due to founder effects during the mass rearing process (Roderick and Navajas 2003).

Four diagnostic mutations were observed between the South Brazil population ('L' and Argentina) and the other populations located in the state of Sao Paulo (N180Y, Y204S, N216Y, V224M). At position 180, the south populations have a leucine (L) (as for *T. urticae* and a T for *P. persimilis*) whereas the remaining specimens have a methionine (M). At position 204, the south populations have a serine (S) (as in *T. urticae* and F in *P. persimilis*) whereas all the remaining specimens have a tyrosine (Y). At position 216, the south

populations have a tyrosine (Y) whereas all the remaining specimens have an asparagine (N) (F in *T. urticae* and S in *P. persimilis*). At position 224, the south populations have a methionine (M) whereas all the remaining specimens have a valine (V) (as in *P. persimilis* and L in *T. urticae*). We can speculate about the meaning of four mutations and their impact on protein function. We have not found examples in the literature of such mutations in mites (nor in arthropods in general). It seems that in insects founder effects and specific geographic locations may lead to accelerated divergence rates in amino acids, or that parasitic life-styles cause an increase in mutation rates (Page et al. 1998). Accelerated rate of Cytb nucleotide and amino acid evolution in bees may be correlated with increased metabolic rates associated with facultative endothermy (Simmon and Weller 2001). Because of the geographic location of *P. macropilis* populations of the two clades (south and São Paulo populations) in two different climates, we can hypothesize that these mutations may be involved in energy used for thermoregulation. Figures 2 and 3 show the structural modification of the Cytb protein between the wild type (São Paulo populations) and mutant types (south populations). Table 2 reports the ΔΔG index (Gibbs free energy). The four mutations have different impacts on the protein stability. It seems, according to the DynaMut model, that mutations N180Y N216Y and V224M have a stabilising effect, whereas mutation Y204S has a destabilizing effect. Changes in binding affinity caused by mutations

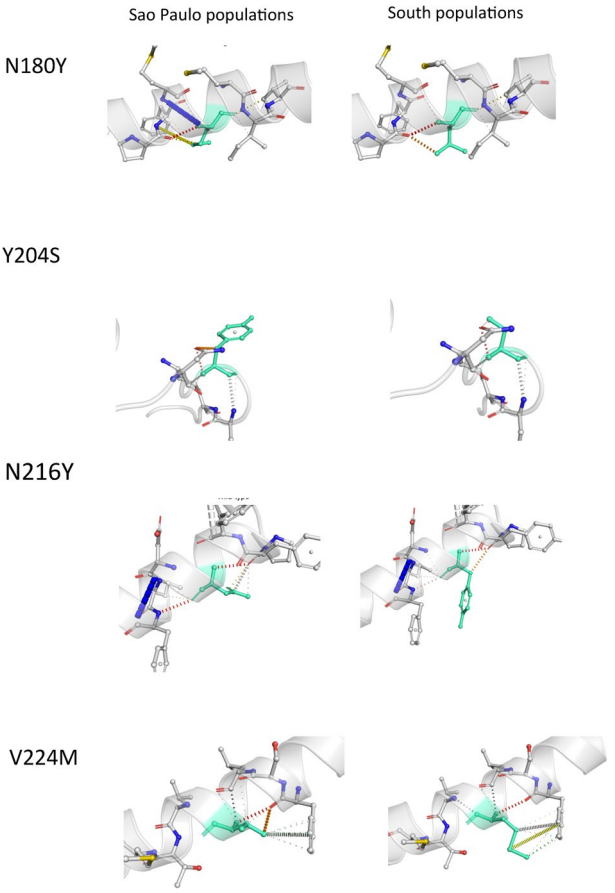

Fig. 3 Prediction of atomic interactions due to mutations N180Y, Y204S, N216Y and V224M in the Cytb protein of *Phytoseiulus macropilis* between populations collected in 'south' (Argentina and Rio Grande do Sul, Brazil) and in Sao Paulo state, Brazil. South and Sao Paulo residues are coloured in light-green and are also represented as sticks alongside with the surrounding residues which are involved in any type of interactions. (Color figure online)

Table 2 ΔΔG index (Gibbs free energy) provided by DynaMut[a] concerning the four diagnostic mutations between the populations of *Phytoseiulus macropilis* (from Sao Paulo state and the south of Brazil and Argentina)

	Mutations			
	N180Y	Y204S	N216Y	V224M
ΔΔG (kcal/mol)	0.181 (stabilizing)	80.689 (destabilizing)	0.112 (stabilizing)	0.562 (stabilizing)

[a]http://biosig.unimelb.edu.au/dynamut/

may affect a molecule's functional activity (Seddigh and Darabi 2018; Geng et al. 2019). There is no literature on Cytb modification stability in arthropods. Several mutations in the human Cytb have been related to diseases. Aledo et al. (2012) compares Cytb stability in mammals. These authors concluded that from the thermodynamic point of view, cytochrome b is much more robust to mutations than COX 1 and stated that more stable proteins can tolerate better a decrease in stability, which in turn allows them to evolve faster. The present study supports these findings, as more stabilizing than destabilizing mutations were observed.

Conclusion

The present paper shows the correct identity of *P. persimilis* and *P. macropilis*, even if lower genetic distances than for other Phytoseiidae species were observed, certainly due to the singular biological features of species of the genus *Phytoseiulus* (inducing rapid evolution rates) and the difficulty to dress a general threshold rule for species differentiation for the whole of the Phytoseiidae family. As stated for insects, it appears that molecular identification should use specific thresholds depending on families, sub-families and even genera concerned. A very low intraspecific variation was observed among the specimens of *P. persimilis*, most likely because of the 'invasion' of the mass-released commercial strains and/or a sampling bias in areas where this species was frequently released. Further comparisons of DNA sequences of *P. persimilis* from South America would permit to test this hypothesis. The intraspecific variation within *P. macropilis* shows population separation mainly according to geographical factors and in a lesser extent according to pesticide resistance. However, additional resistant populations should be tested to determine whether such a separation is not due to other factors, especially founder and drift effects (Roderick and Navajas 2003), as the resistant population is laboratory-reared for 10 years. Furthermore, no diagnostic mutation in amino acid sequence was observed. On the opposite, four mutations were identified between geographically distant *P. macropilis* populations. This finding clearly opens new research lines on the effect of mutations on protein functioning in mites. It is the first time that such mutations are reported and that stability of proteins after mutation is investigated. However, much more work is required, especially for better characterizing the biological features of the populations (for instance, in relation to temperature requirements) and to better assess the functional positive or negative impact of the mutations in relation to thermodynamic characteristics of the protein.

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Declarations

Conflict of interest There is no conflict of interest concerning the results provided in this manuscript. The authors declare no conflicts of interest.

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