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Drivers for mutation in amino acid sequences of two mitochondrial proteins (Cytb and COI) in Phytoseiidae mites (Acari: Mesostigmata)

Marie-Stéphane Tixier[1] · Lou Tabary[1] · Martial Douin[1]

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Abstract
Mutations in amino acid sequences can affect protein function. Such aspects have been poorly studied for arthropods. As recent studies have shown mutations in cytochrome b (Cytb) associated with geographic locations in several Phytoseiidae species, the present study aims at investigating (i) the mutation pattern in additional species for the Cytb fragment, (ii) the mutation pattern for another mitochondrial amino acid sequence, cytochrome c oxidase subunit 1 (COI), and (iii) factors affecting the mutations observed (taxonomy, plant support, climatic variables, wild vs. commercialised species). Mutations in amino acid sequences were assessed in seven Phytoseiidae species, with populations collected in contrasted environments. The DNA sequences were mainly obtained from published studies and some were newly obtained. Mutations were observed within and between the populations considered for both fragments, with higher mutation rates in Cytb than in COI sequences, confirming the robustness of this former fragment. Plant support and taxonomic position were not related to mutation patterns. A lower number of mutations was observed in commercialised populations than in wild ones. As preliminary tendencies, mutations in Cytb and COI sequences seem associated to temperature and moisture. Such a preliminary approach, attempting to relate mutation to functional adaptations, clearly opens new research tracks for better assessment of the drivers of mite adaptation, in a context of climate change.

Keywords Protein · Mutations · Phytoseiid mites · Temperatures · Precipitations · Water vapour pressure

Introduction

Molecular approaches using DNA sequences greatly increase since a few decades, especially for population genetics and phylogenetic issues. Recently, researches focus not only on DNA diversity but also on amino acid composition (e.g., Young and Hebert 2015; Pentinsaari et al.

✉ Marie-Stéphane Tixier
marie-stephane.tixier@supagro.fr

[1] CBGP, Institut Agro Montpellier, INRAE, CIRAD, IRD, University Montpellier, 755 Avenue du Campus Agropolis, CS 30016, 34988 Montferrier-sur-Lez cedex, France

2016; Khalifa et al. 2018). Whereas the impact of amino acid changes on protein function is well studied in vertebrates (e.g., mammals, fishes), especially for human health, those aspects are poorly investigated for arthropods (i.e., Camps et al. 2007; Somero 2010; Pappalardo et al. 2015). The scarce studies on insects and mites essentially focus on the impact of amino acid mutations in insecticide targets (i.e., acetylcholinesterase, ATP-ase, Cytb mtDNA) (i.e., Van Leeuwen et al. 2011; Dobler et al. 2012; Douris et al. 2016) and in heat shock proteins (Hsp) for climate warming adaptation, especially in *Drosophila melanogaster* Meigen (Hoffman et al. 2003; Hoffman and Willi 2008). Recently, studies on several species of Phytoseiidae mites—*Typhlodromus* (*Anthoseius*) *recki* Wainstein, *Phytoseiulus macropilis* (Banks), *Typhlodromus* (*Typhlodromus*) *phialatus* Athias-Henriot, and *Typhlodromus* (*Anthoseius*) *rhenanoides* Athias-Henriot—suggest that mutations in the amino acid sequences of the Cytb mtDNA are associated with adaptation to geographical locations (Queiroz et al. 2021; Tixier et al. 2021). Such results clearly open new avenues to determine / predict population adaptation to different constraints (Pörtner 2002).

Phytoseiidae mites are predators, largely used in biological control of pest mites and small insects all over the world (i.e., Gerson et al. 2003; McMurtry et al. 2013; Demite et al. 2020). They show diverse feeding habits, with most of the species being generalists and some specialists on their prey (McMurtry et al. 2013; Tixier 2018). Investigating relationships between DNA variation, amino acid mutations and functional aspects can provide knowledge on the drivers of Phytoseiidae evolution. Such knowledge might be useful for biological control applications especially in an early selection of adapted populations / species to specific conditions (i.e., drought). Even if mutations in the acid amino sequence of Cytb mtDNA seem to be associated to geographical locations, conclusions based on a few species cannot provide a general rule. Furthermore, no attempt to relate climatic factors to differentiation was carried out until now (Queiroz et al. 2021; Tixier et al. 2021).

The two proteins herein considered (Cytb and COI mitochondrial fragments) belong to the cytochrome C oxidase complex involved in electron transport, dioxygen reduction and proton pumping in the respiratory chain of mitochondria (e.g., Degli Espoti et al. 1993; Tsukihara et al. 1996). Thus, mutations in the amino acid sequence may affect mite metabolic performance and adaptation to particular environmental conditions. As the number of COI and Cytb mtDNA sequences is increasing for Phytoseiidae (because of diagnosis and phylogenetic aspects), the objective here is to analyse these datasets to assess the occurrence of mutations and their relation to ecological factors as climatic conditions, plant supports and taxonomy. Furthermore, as some Phytoseiidae species are commercialised and mass-released in crops whereas others occur naturally (McMurtry et al. 2013; Tixier 2018), higher diversity is expected in wild populations than in commercialised ones.

Furthermore, several studies on insects report a purifying selection on COI mtDNA and Cytb mtDNA, because of their functional importance (Simmons and Weller 2001; Meiklejohn et al. 2007; Pentinsaari et al. 2016; Sabir et al. 2019). For mites, such studies are scarce. Brandt et al. (2017) showed more effective purifying selection despite the lack of sex across Oribatida lineages, considering 10 orthologous mitochondrial genes (atp6, cob, cox1, cox2, cox3, nd1, nd2, nd3, nd4 and nd5) and comparing asexual and sexual taxa. Using the two datasets for COI and Cytb mtDNA, here we also evaluate purifying selection in Phytoseiidae.

Materials and methods

Species and populations considered

The species considered, the locations where the populations were collected and GenBank accession numbers are shown in Table 1. We considered different species for the two proteins studied, because no congruent datasets in both DNA fragments exist. In both cases, we chose species with a sufficient number of specimens per population: the number of specimens is > 10 except for *T.* (*A.*) *recki* for which only five specimens were available for the COI DNA fragment. We also considered populations collected in contrasted climatic areas, and/or with contrasted biological control features (commercial vs. wild populations, see Table 1). Species considered belong to the three sub-families of the family Phytoseiidae.

For the Cytb mtDNA analyses, five species were considered, three commonly commercialised ones: *Neoseiulus californicus* (McGregor), *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius swirskii* Athias-Henriot (sub-family: Amblyseiinae) and two non-commercialised species: *Typhlodromus* (*Typhlodromus*) *pyri* Scheuten (Typhlodrominae) and *Kampimodromus aberrans* (Oudemans) (Amblyseiinae). For the COI mtDNA fragment, five species were considered, two commonly commercialised ones – *P. persimilis*, *A. swirskii* – and three non-commercialised species: *Phytoseius finitimus* Ribaga (Phytoseiinae), *T.* (*A.*) *recki* and *T.* (*T.*) *pyri* (Typhlodrominae).

DNA analyses

The two molecular markers considered are mitochondrial DNA fragments (Cytb and COI), currently used for species diagnosis in Phytoseiidae (e.g., Dos Santos and Tixier 2017). In the present work, we mainly used the DNA sequences already obtained (by ourselves) and published in studies focusing on molecular identification (Table 1). For some specimens, DNA sequences were herein obtained following the protocols used by Kanouh et al. (2010) for DNA extraction and Tixier et al. (2012) for PCR conditions. The nucleotide composition and transition / transversion rates were calculated using MEGA following the MCL substitution matrix model (Kumar et al. 2018).

Amino acid analyses

The amino acid sequences of the partial Cytb and COI mtDNA fragments were obtained using https://web.expasy.org/translate/. The total amino acid sequence of one specimen of *Tetranychus urticae* Koch retrieved from GenBank (YP_001795379) was aligned (using MEGA) with the Phytoseiidae amino acid sequences to determine common mutations and refer to universal positions. We used *T. urticae* sequence as in Tixier et al. (2021) and Queiroz et al. (2021) for assessing the mutation positions (Table 2).

Some mutations were present in all the specimens of a single population, but it was not the case for all populations. However, if one mutation was dominant in a population (occurring in > 70% of the specimens), we considered this mutation as 'characteristic' of the population. For the study of mutation occurrence within populations, mutations were considered variable, if at least one specimen per population carries this mutation. Swiss-model (https://swissmodel.expasy.org/) and DynaMut (http://biosig.unimelb.edu.au/dynamut/) online tools (Rodrigues et al. 2018) were used for assessing mutation impact on the

Table 1 Collection localities and plant supports of the phytoseiid populations considered, and accession numbers of the DNA sequences of COI and cytb mtDNA in GenBank

DNA fragment	Species	Country	Locality	Host plant	Assession numbers
Cytb	*Amblyseius swirskii*	Koppert	Commercial strain from France	–	**MT828779, MT828780, MT828781, MT828782, MT828783**
			Commercial strain from Spain	–	**MT828797, MT828798, MT828799, MT828800, MT828801**
		Syngenta	Commercial strain	–	**MT828784**
		France Reunion island	Montvert (55°32′19"S, 21°19′42"E)	*Capsicum annuum* (Solanaceae)	**MT828785, MT828786, MT828787, MT828788, MT828789, MT828790, MT828791, MT828792, MT828793**
		Israel	Not known	Not known	**MT828794, MT828795, MT828796**
		Capo Verde	Ribeira da Torre (17°8′13"N, 25°3′58"W)	*Ageratum conyzoides* (Asteraceae)	**MT828802, MT828803, MT828804, MT828805, MT828806, MT828813, MT828818**
		Benin	Brazilian population issued from Israel	Not known	**MT828807, MT828814, MT828815, MT828816, MT828817**
			Idigny (7°28′19.7"N, 2°41′01.8"E)	*Solanum macrocarpon* (Solanaceae)	**MT828808, MT828809, MT828810, MT828811, MT828812**
		Egypt	Al-Azizia, Sharqia Governorate (30°02′44.6"N, 31°10′56.0"E)	*Citrus* sp. (Rutaceae)	**MT828819, MT828820, MT828821, MT828822, MT828823, MT828824, MT828825, MT828826, MT828827, MT828828, MT828829, MT828830**

Table 1 (continued)

DNA fragment	Species	Country	Locality	Host plant	Assession numbers
	Kampimodromus aberrans	Austria	Vienna (48°12′28.77"N, 16°22′24.57"E)	*M. domestica*	**MT828748, MT828749**
		France	Montpellier (43°36′38.70"N, 3°52′36.05"E)	*Celtis australis* (Cannabaceae)	**MT828750, MT828751, MT828752, MT828753**
			Marsillargues (43°39′54.81"N, 4°10′41.99"E)	*M. domestica*	**MT828754, MT828755, MT828756, MT828757**
			Pouzolles (43°28′34.64"N, 3°16′38.62"E)	*Quercus pubescens* (Fagaceae)	KU318205, KU318206, **MT828758, MT828759, MT828760, MT828761**
			Restinclieres (43°43′2.84"N, 3°51′35.95"E)	*Vitis vinifera* (Vitaceae)	**MT828767, MT828768, MT828769, MT828770, MT828771, MT828772 MT828773, MT828774**
			Near Brissac (43°52′07"N, 3°41′47"E)	*Q. pubescens*	**MT828775**
		Croatia	Veprinac (45°20′8.96"N, 14°16′30.69"E)	*Corylus avellana* (Betulaceae)	**MT828762, MT828763, MT828764, MT828765, MT828766**
		Italy	Padova (45°24′26.73"N, 11°52′49.89"E)	*V. vinifera*	GU591694, GU591693
	Neoseiulus californicus	South Korea	Jeju (33°29′58.63"N, 126°31′52.25"E)	*Citrus reticulata*	JF279241, JF279242, JF279243, JF279244, JF279245, JF279246, JF279247, JF279248
		Chile	La Cruz (32°49′32.64"S, 71°13′37.04"W)	*Phaseolus vulgaris* (Leguminosae)	JF279201, JF279202, JF279203
		South Africa	Pretoria (25°43′41.80"S, 28°13′5.11"E)	*Malus domestica* (Rosaceae)	JF279235, JF279236, JF279237, JF279238, JF279239, JF279240

Table 1 (continued)

DNA fragment	Species	Country	Locality	Host plant	Assession numbers
		Tunisia	Tozeur (33°55′26.75"N, 8°7′52.53"E)	*Convolvulus arvensis* (Convolvulaceae)	JF279209, JF279210
			Cap Bon (37°4′60.00"N, 11°2′20.00"E)	*Solanum nigrum*	**MT862740, MT862741, MT862742, MT862743, MT862744, MT862745 MT862746**
		Spain	Valencia (39°29′31.08"N, 0°21′20.95"W)	*Fragaria vesca* (Rosaceae)	JF279211, JF279212, JF279213, JF279214, JF279215
		Greece	Thessaloniki (40°38′21.66"N, 22°56′40.58"E)	*P. vulgaris*	JF279225, JF279226, JF279227, JF279228, JF279229
		Italy	Firenze (43°46′11.85"N, 11°14′51.48"E)	*F. vesca*	JF279221, JF279222, JF279223, JF279224
			Sicily (38°7′0.28"N, 13°21′39.19"E)	*F. vesca*	JF279216, JF279217, JF279218, JF279219, JF279220
			Rearing units	–	**MT828737, MT828738, MT828739, MT828740, MT828741**
		Brazil	Piracicaba (22°43′29.83"S, 47°38′51.27"W)	*P. vulgaris*	JF279204, JF279205, JF279206, JF279207, JF279208
		Japan	Ibaraki (36°20′30.52"N, 140°26′48.44"E)	*P. vulgaris*	JF279230, JF279231, JF279232, JF279233, JF279234
		Koppert	Commercial rearings	–	JF279193, JF279194, JF279195, JF279196
		Israel	Not known	-	**MT828736**
		USA	New York (40°42′51.67"N, 74°0′21.50"W)	*M. domestica*	JF279197, JF279198, JF279199, JF279200

Table 1 (continued)

DNA fragment	Species	Country	Locality	Host plant	Assession numbers
		France	Marsillargues (43°39′54.81"N, 4°10′41.99"E)	*M. domestica*	JF279187, JF279188
			Villeneuve les Maguelones (43°30'N, 3°53'E)	Fabaceae	**MT828742**
			Miomo (Corsica) (42°4437.85″N, 9°27′39.81″E)	*S. nigrum*	**MT828743, MT828744, MT828745, MT828746, MT828747**
			Mauguio (43°36′55.63"N, 4° 0′36.58"E)	*Solanum melongena*	JF279184, JF279185, JF279186, **MT828735**
			Midi-Pyrénées (44°5′9.39"N, 1°31′15.10"E)	*M. domestica*	JF279189, JF279190, JF279191, JF279192
	Phytoseiulus persimilis	Koppert	Commercial strain	–	FJ985083, FJ985084, FJ985085
		France	Montpellier (43°36′38.70"N, 3°52′36.05"E)	*P. vulgaris*	FJ985069, FJ985070, FJ985071, FJ985072, FJ985073, FJ985074, FJ985075
			Cabannes de Salaison (43°35'N, 4°00'E)	*Broussonetia papyrifera* (Moraceae)	**MT110297**
		Spain	Valencia (39°29′31.08"N, 0°21′20.95"W)	Not known	FJ985076, FJ985077, FJ985078, FJ985079, FJ985080
		Italy	Not known	Not known	GU232492, GU232493
		Tunisia	Not known	Not known	GU232494, GU232495, GU232496, GU232497
			Cap Bon (37°4′60.00"N, 11°2′20.00"E)	*S. nigrum*	**MT110298**
				Amaranthus reflexus (Amaranthaceae)	**MT110299, MT110300**

Table 1 (continued)

DNA fragment	Species	Country	Locality	Host plant	Assession numbers
	Typhlodromus (*T.*) *pyri*	France	Restinclieres (43°43′2.84"N, 3°51′35.95"E)	*Rubus* sp. (Rosaceae)	**MT828776, MT828777**
			Valleraugue (44° 4′49.04"N, 3°38′27.88"E)	*Rubus* sp.	JF279266, JF279267, JF279268, JF279269, **MT828778**
			Mercantour (43°98 N, 07°55E)	*Cornus sanguinea* (Cornaceae)	JF279283, JF279284, JF279285
			Burgundy (47°2′30.61"N, 4°22′23.31"E)	*V. vinifera*	JF279274, JF279275, JF279276, JF279277, JF279278, JF279279, JF279280, JF279281, JF279282
		Belgium	Not known	*Malus* sp.	JF279251
		Austria	Vienna (48°12′28.77"N, 16°22′24.57"E)	*V. vinifera*	JF279252, JF279253, JF279254
		Italy	Padova (45°24′26.73"N, 11°52′49.89"E)	*V. vinifera*	JF279257, JF279258, JF279259, JF279260
		Poland	Not known	*Malus* sp.	JF279255, JF279256
		USA	New York (40°42′51.67"N, 74°0′21.50"W)	*M. domestica*	JF279270, JF279271, JF279272, JF279273
		Hungary	Raposka (46°50′56.58"N, 17°25′34.62"E)	*V. vinifera*	JF279261, JF279262, JF279263, JF279264, JF279265

Table 1 (continued)

DNA fragment	Species	Country	Locality	Host plant	Assession numbers
COI	*Amblyseius swirskii*	Koppert	Commercial strain from France	–	**MW074349, MW074350, MW074351**
		France Reunion island	Montvert (55°32′19"S, 21°19′42"E)	*C. annuum*	**MT827876, MT827877, MT827878, MT827879, MT827880, MT827881, MT827882, MT827883, MT827884**
		Israel	Not known	Not known	**MW074353, MW074354, MW074355, MW074356**
		Koppert	Commercial strain from Spain	–	**MT827885, MT827886, MT827887, MT827888, MT827889**
		Capo Verde	Ribeira da Torre (17°8′13"N, 25°3′58"W)	*A. conyzoides*	**MT827894, MT827895, MT827896, MT827897, MT827898, MT828365**
		Benin	Brazilian population issued from Israel	Not known	**MT827915, MT827916, MT827917, MT827918, MT827919**
			Idigny (7°28′19.7"N, 2°41′01.8"E)	*S. macrocarpon*	**MT819959, MT819960, MT819961, MT819962**
		Egypt	Al-Azizia, Sharqia Governorate (30°02′44.6"N, 31°10′56.0"E)	*Citrus* sp.	**MT827899, MT827900, MT827901, MT827902, MT827903, MT827904**
		Syngenta	Commercial strain	–	**MW074352**

Table 1 (continued)

DNA fragment	Species	Country	Locality	Host plant	Assession numbers
	Phytoseiulus persimilis	Tunisia	Cap Bon (37°4′60.00"N, 11°2′20.00"E)	*S. nigrum*	**MW074343**
		France	Montpellier (43°36′38.70"N, 3°52′36.05"E)	*P. vulgaris*	**MW074344**
		Spain	Valencia (39°29′31.08"N, 0°21′20.95"W)	Not known	**MW074339, MW074340, MW074341**
		Koppert	Commercial strain	–	**MW074345, MW074346, MW074347**, KU318199, KU318200
	Phytoseius finitimus	Italy	Padova (45°55′52.16″N, 12°16′11.63″E)	*V. vinifera*	KX021146, KX021147, KX021148, KX021149, KX021150, KX021151, KX021152, KX021153, KX021154, KX021155, KX021156, KX021157, KX021158
				Viburnum tinus (Adoxaceae)	KX021132, KX021133, KX021134, KX021135, KX021136, KX021137, KX021138
		France	Corsica (42°16′53’’N, 9°32′2’’E)	*Actinidia deliciosa* (Adoxaceae)	KX021128, KX021129, KX021130, KX021131, **MT827905, MT827906, MT827907, MT827908, MT827909, MT827910, MT827911, MT827912, MT827913, MT827914**

Table 1 (continued)

DNA fragment	Species	Country	Locality	Host plant	Assession numbers
	Typhlodromus (*Anthoseius*) *recki*	France	Montferrier-sur-Lez Bailarguet (43°40′57.5″N, 3°52′25.2″E)	Not known	**MT828361, MT828362**
		Italy	Ficuzza (Palermo) (37°53′18.7″N, 13°23′27.3″E)	*Cirsium arvense* (Compositae)	**MT828363, MT828364, MW074348**
	Typhlodromus (*T.*) *pyri*	France	Burgundy (47°2′30.61"N, 4°22′23.31"E)	*V. vinifera*	JF279156, JF279157, JF279158, JF279159, JF279160, JF279161
			Valleraugue (44° 4′49.04"N, 3°38′27.88"E)	*Rubus* sp.	JF279162, JF279163, JF279164, JF279165
			Mercantour (43°98 N, 07°55E)	*C. sanguinea*	JF279169, JF279170, JF279171
		USA	New York (40°42′51.67"N, 74°0′21.50"W)	*M. domestica*	JF279166, JF279167, JF279168
		Austria	Vienna (48°12′28.77"N, 16°22′24.57"E)	*V. vinifera*	JF279172, JF279173, JF279174
		Hungary	Raposka (46°50′56.58"N, 17°25′34.62"E)	*V. vinifera*	JF279175, JF279176, JF279177, JF279178
		Italy	Padova (45°24′26.73"N, 11°52′49.89"E)	*V. vinifera*	JF279179, JF279180
		Poland	Not known	*Malus* sp.	JF279181

The accession numbers for the DNA sequences acquired for the present study are in bold type, those deposited by the authors (published work) are in roman type

Table 2 Nucleotide frequencies (%) in the Cytb and COI mtDNA fragments for the Phytoseiidae species herein considered

Marker	Species	Nucleotide frequency (%)			
		A	T(U)	G	C
Cytb	*Amblyseius swirskii*	33.6	38.2	7.9	20.3
	Kampimodromus aberrans	31.9	37.7	8.2	22.2
	Neoseiulus californicus	29.8	40.5	9.4	20.3
	Phytoseiulus persimilis	36.15	39.38	6.49	17.97
	Typhlodromus (*T.*) *pyri*	28.3	43.8	11	16.9
COI	*A. swirskii*	25.39	43.74	13.02	17.34
	P. persimilis	26.22	42.61	13.79	17.38
	Phytoseius finitimus	31.94	37.84	17.59	12.62
	Typhlodromus (*Anthoseius*) *recki*	23.37	43.20	13.77	19.66
	T. (*T.*) *pyri*	26.22	39.64	20.10	14.04

protein structure and function. The ΔΔG index (Gibbs free energy) was assessed to characterise mutation impact on protein stability and dynamics.

For the species and the DNA fragments for which a sufficient number of populations were considered and a significant number of mutations between the populations was observed, principal component analyses (PCA) were carried out to identify for each species relationships between population clusters and mutations. For this, a matrix was constructed: the rows are the populations, the identified mutations are the columns and in each cell we noted the percentage of specimens within the population carrying the mutations. Fourteen climatic variables (see below) were considered as supplementary factors to determine any correlation between mutations and climatic conditions. First, we used factors associated to temperature: (i) the mean global temperature (°C) per year, (ii) mean temperature of the driest quarter, (iii) mean temperature of the wettest quarter, (iv) mean temperature of the warmest quarter, (v) mean temperature of the coldest quarter, (vi) mean temperature of the wettest month, and (vii) mean temperature of the driest month. Second, we considered precipitation (mm) variables: (i) annual precipitation, (ii) precipitation of the driest quarter, (iii) precipitation of the wettest quarter, (iv) precipitation of the warmest quarter, and (v) precipitation of the coldest quarter. Water vapour pressure (kPa) and altitude were also considered. Climatic variables and water vapour pressure (kPa) were retrieved from World Clim v.2 at 10 min resolution (ca. 340 km^2). The averages (from 1970 to 2000) were extracted using R software and Geotiff files for each variable considered (Raster/map packages). Altitude was retrieved from https://www.coordonnees-gps.fr/, based on GPS coordinates of the locations where mites were collected. Multifactorial analyses were performed using R package FactoMineR v.2.3 (R Core Team 2020).

To estimate the non-synonymous / synonymous substitution rates (dN/dS), the amino acid sequences were aligned and the mixed effects model of evolution (MEME) model was applied using the on-line tool http://www.datamonkey.org/meme, to detect any sites subject to episodic diversifying or positive selection (Murrell et al. 2012). If the ratio $dN/dS > 1$, the sequence is considered under positive selection, with an overabundance of non-synonymous substitutions encouraging a sweep of new beneficial alleles. When the ratio $dN/dS < 1$, purifying selection is inferred. For the Cytb analysis, amino acid sequences already published for the species *T.* (*A.*) *recki*, *P. macropilis*, *P. finitimus*, *T.* (*A.*) *rhenanoides*, and

T. (*T.*) *phialatus* (Tixier et al. 2021; Queiroz et al. 2021) were included in the analysis to increase the dataset analysed.

Results and discussion

Overall data description

The Cytb fragments considered range between 399 and 414 bp, and the COI mtDNA fragments from 549 and 1,059 bp (Table 4). For both fragments and all species, the percentage of A-T is the highest, which agrees with previous results on arthropod mitochondrial DNA (Simmons and Weller 2001; Albu et al. 2008). Table 3 shows the substitution rates between each nucleotide for both fragments. A similar pattern is observed for all species and both fragments. Higher transition than transversion rates are observed.

Table 4 shows the number of variable sites for each species and each DNA fragment. For the Cytb mtDNA, the percentage of variable sites ranges between about 17% (for *N. californicus* and *T.* (*T.*) *pyri*) and 1.25% (for *P. persimilis*). No relation between this variation and the number of specimens and populations considered per species was clearly observed, even if the highest variation was observed for the two species for which the highest number of populations was considered. For the COI mtDNA fragment, the percentage of variable sites ranges between 1.64% (*T.* (*A.*) *recki*) and about 15% (*T.* (*T.*) *pyri*, *P. finitimus*). It seems that high variation is associated with species for which the number of specimens considered was the highest. However, it is not the only explanation as for *A. swirskii*, with many specimens studied, only 4.15% of variable sites were noted. The variation in *T.* (*T.*) *pyri*, *P. persimilis* and *A. swirskii* shows the same trend for both DNA fragments (low variation for *A. swirskii* and *P. persimilis*, high variation for *T.* (*T.*) *pyri*). Generally, for all species, less variation was observed for the COI than for the Cytb mtDNA fragment, which agrees with previous results on intraspecific variation of these two markers in Phytoseiidae species (i.e., Dos Santos and Tixier 2017).

The percentage of variable sites in the amino acid sequence for Cytb mtDNA (Table 4) ranges between 1.5% (*P. persimilis*) and 21.4% (*N. californicus*). These values are higher than the variation rates observed at DNA level, except for *T.* (*T.*) *pyri*. For the COI mtDNA, the percentage of variable sites ranges between 2.2% (*P. persimilis*) and 8.8% (*P. finitimus*). Generally, these rates are lower than the ones observed at DNA level, suggesting that most DNA mutations in the COI fragment are probably silent at the protein level.

Figure 1 shows the proportion of each amino acid and their biochemical properties in both protein fragments, for all the species considered. The two protein sequences consist mainly in non-polar amino acids whatever the species considered. Such results are consistent with other data on insects, stating that for membrane-embedded protein, a large majority of non-polar amino acids is expected (Pentinsaari et al. 2016; Sabir et al. 2019). The second main category is polar uncharged amino acids, the three other types (aromatic, positive and negative) being less frequently observed. Some amino acids are frequent, especially isoleucine, leucine, methionine (non-polar), proline, serine (polar uncharged) and phenylalanine (aromatic). Similar amino acid compositions are observed among the species considered, even if the Cytb sequence of *P. persimilis* shows higher rates of alanine and glycine than in the other species. Figure 2 shows the position of variable amino acids along the two protein fragments for each species herein considered and other species (data retrieved from Tixier et al. (2021) for Cytb fragment). For both Cytb and COI sequences,

Table 3 Nucleotide substitution patterns (%) for the Phytoseiidae species considered and the two mtDNA fragments (Cytb and COI), calculated using MEGA following the MCL substitution matrix model

Species		Cytb				COI			
		A	T	C	G	A	T	C	G
Amblyseius swirskii	A	–	6.72	3.63	4.58	–	2.35	0.7	13.22
	T	5.91	–	14.05	1.34	1.37	–	13.15	0.95
	C	5.91	26.02	–	1.34	1.37	43.89	–	0.95
	G	20.17	6.72	3.63	–	19	2.35	0.7	–
	Transition rates	64.82				89.26			
	Transversion rates	35.18				10.74			
Kampimodromus aberrans	A	–	3.43	2	8.3				
	T	2.89	–	15.11	0.73				
	C	2.89	25.86	–	0.73				
	G	32.63	3.43	2	–				
	Transition rates	81.9							
	Transversion rates	18.1							
Neoseiulus californicus	A	–	5.53	2.76	6.74				
	T	4.06	–	14.83	1.27				
	C	4.06	29.72	–	1.27				
	G	21.48	5.53	2.76	–				
	Transition rates	72.77							
	Transversion rates	27.23							
Phytoseiulus persimilis	A	–	9.59	4.38	5.32	–	2.67	0.86	13.95
	T	8.8	–	5.13	1.58	1.64	–	12.79	1.09
	C	8.8	11.24	–	1.58	1.64	39.72	–	1.09
	G	29.61	9.59	4.38	–	21.02	2.67	0.86	–
	Transition rates	51.3				87.48			
	Transversion rates	48.7				12.52			
Phytoseius finitimus	A					–	1.26	0.59	14.66
	T					1.06	–	13.07	0.41
	C					1.06	28.16	–	1.41
	G					37.47	1.26	0.59	–
	Transition rates					93.36			
	Transversion rates					6.64			
Typhlodromus (*Anthoseius*) *recki*	A					–	2.9	0.93	34.3
	T					1.58	–	2.51	1.29
	C					1.58	7.89	–	1.29
	G					41.9	2.9	0.93	–
	Transition rates					86.6			
	Transversion rates					13.4			
Typhlodromus (*T.*) *pyri*	A	–	3.4	1.26	14.3	–	2.05	1.05	8.92
	T	2.21	–	8.64	0.82	1.36	–	21.58	0.73
	C	2.21	23.27	–	0.82	1.36	42.38	–	0.73
	G	38.4	3.4	1.26	–	16.74	2.05	1.05	–
	Transition rates	84.61				89.62			
	Transversion rates	15.39				10.38			

Table 4 Number of populations and specimens considered for each phytoseiid species, number of nucleotides and amino acids and percentage of variable sites for DNA and amino acid sequences of Cytb and COI markers

Marker	Species	N° specimens	N° populations	DNA sequence			Protein sequence		
				N° nucleotides	N° variable sites	% variable sites	N° amino acids	N° variable sites	% variable sites
Cytb	*Amblyseius swirskii*	53	9	405	26	6.42	80	10	12.50
	Kampimodromus aberrans	32	8	405	42	10.37	112	16	14.29
	Neoseiulus californicus	85	20	405	69	17.04	117	25	21.37
	Phytoseiulus persimilis	26	8	399	5	1.25	133	2	1.50
	Typhlodromus (T.) pyri	38	10	414	72	17.39	134	16	11.94
COI	*A. swirskii*	43	9	1059	44	4.15	353	9	2.55
	P. persimilis	10	4	675	15	2.22	225	5	2.22
	Phytoseius finitimus	33	3	651	100	15.36	217	19	8.76
	Typhlodromus (Anthoseius) recki	5	2	669	11	1.64	223	8	3.59
	T. (T.) pyri	26	8	549	83	15.12	183	8	4.37

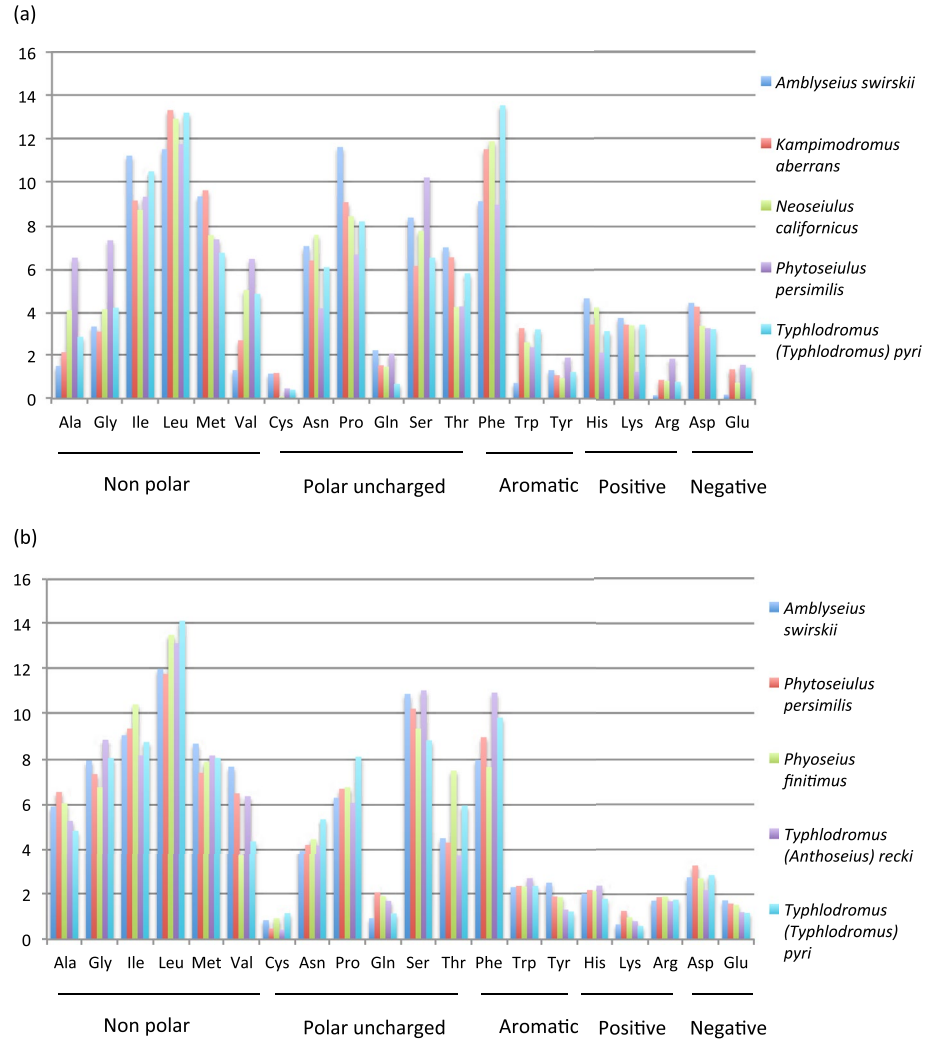


Fig. 1 Average amino acid frequencies in Cytb (**a**) and COI (**b**) fragments for the Phytoseiidae species *Neoseiulus californicus*, *Amblyseius swirskii*, *Typhlodromus* (*T.*) *pyri*, *Kampimodromus aberrans*, *Phytoseiulus persimilis*, *Typhlodromus* (*Anthoseius*) *recki* and/or *Phytoseius finitimus*. The amino acids are grouped by their biochemical properties as in Pentinsaari et al. (2016)

mutations are distributed all along the fragments. No similarity in mutation distributions between the species / sub-families considered was observed.

Table 5 shows the dN/dS ratio for each species and both markers. For the Cytb and COI fragments considering 10 and five Phytoseiidae species, respectively, the dN/dS ratio is < 1 (Cytb: 0.188; COI: 0.0585). All these results suggest purifying selection (more synonymous mutations than non-synonymous ones), tending to limit the DNA mutations impacting protein function, in accordance with the key role of these two proteins involved in electron transport during the respiration process (Degli Espoti et al. 1993; Tsukihara et al. 1996). Those results also agree with previous analyses stating that there is a prevalence

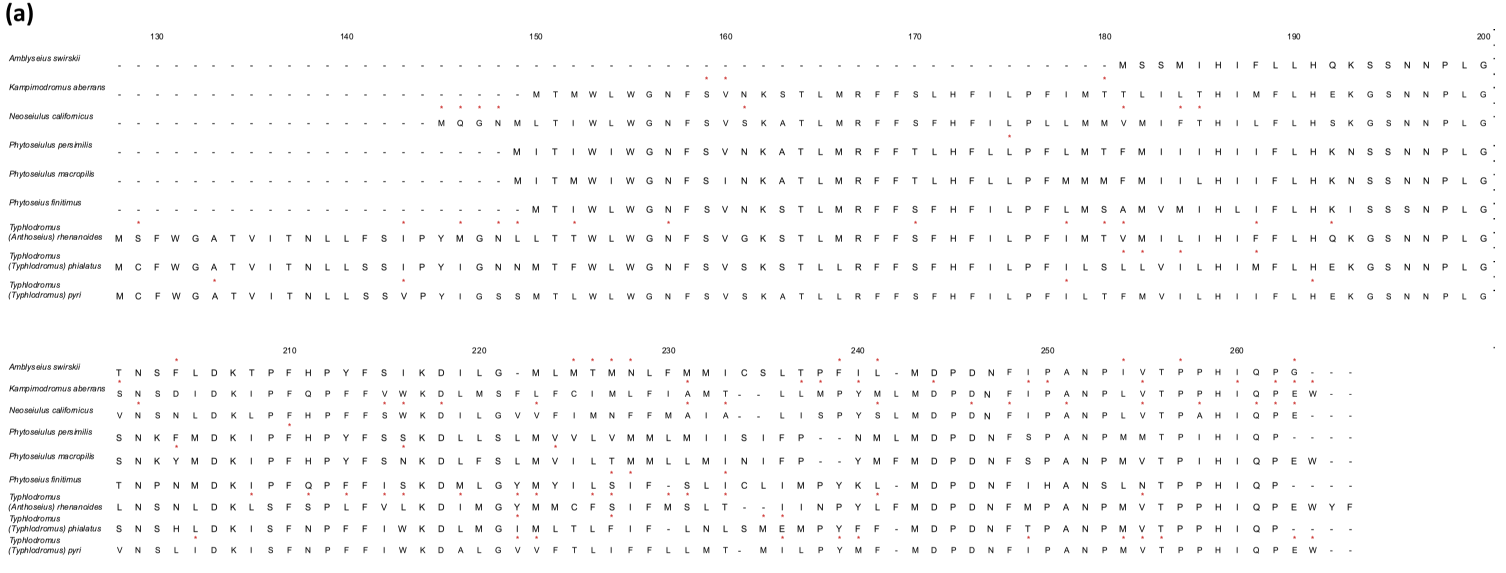


Fig. 2 Alignment of the amino acid sequences of Cytb (**a**) and COI (**b**) fragments. Red stars correspond to mutation occurrence between and within populations for each species considered

Fig. 2 (continued)

Table 5 dN/dS ratio obtained using MEME (http://www.datamonkey.org/meme) for the Cytb and COI sequences for the Phytoseiidae species considered

Species	dN/dS Cytb	dN/dS COI
Amblyseius swirskii	0.194	0.0442
Kampimodromus aberrans	0.208	–
Neoseiulus californicus	0.158	–
Phytoseiulus persimilis	0.119	0.215
P. macropilis	0.174	–
Phytoseius finitimus	0.0828	0.0352
Typhlodromus (*Anthoseius*) *recki*	0.593	0.899
T. (*A.*) *rhenanoides*	0.275	–
T. (*T.*) *phialatus*	0.157	–
T. (*T.*) *pyri*	0.0678	0.0141

of purifying selection in mtDNA (Meiklejohn et al. 2007; Galtier et al. 2009). The dN/dS ratio is lower for COI than for the Cytb sequences, perhaps because we considered a lower number of specimens, or because of the lower robustness of this fragment, requiring higher purifying selection to avoid deleterious mutations. These results are similar to those reported in previous studies that demonstrated that COX genes seem to have lower rates of divergence than Cytb gene (Monthooth unpubl., in Meiklejohn et al. 2007).

Cytb amino acid variation between populations within species

Neoseiulus californicus

Fourteen mutations are observed among the 20 populations of *N. californicus* considered (Table 6). Six mutations separate the populations Marsillargues, Midi-Pyrénées and Villeneuve-les-Maguelone from the 17 others. It seems that these mutations separate 'commercial' populations (introduced in various countries) and 'wild' populations collected in France (except France-Corsica assumed to be wild). Four of these mutations have a stabilizing effect (S161N, V181M, V223L and A233V) and two have a destabilizing effect (F184I, T185I). Three mutations decrease molecule flexibility (S161N, V181M, A233V), whereas three increase it (F184I, T185I, V223L). Mutation effects on vibrational Entropy Energy and interatomic interactions are shown in the supplementary files 1 and 2.

Other mutations, separating one population from the others, are also observed (Greece S215F, USA W216S, Corsica M145Y, Q146T, Italy rearings A231T). The unique specimen from Villeneuve-les-Maguelone is cumulating four prevailing mutations.

The amino acid mutation (aam) pattern of the multifactorial analysis (Fig. 3a) shows no differentiation among the 'commercial' (all grouped in the centre of the graph). 'Wild' populations are well separated from the 'commercial' ones but also from each other, especially along axis 2, except from Midi-Pyrénées and Marsillargues populations grouped together. No clear relation between climatic variables and mutations is noted probably because the commercial populations are grouped together whatever the localities where they have been collected. The aam pattern of the four 'wild' populations (Corsica, Marsillargues, Midi-Pyrénées and Villeneuve-Les-Maguelone) seems to be slightly related to one climatic variable (mean temperature of the wettest quarter) along axis 1 (higher temperature in Corsica and Villeneuve-Les-Maguelone than in the other two localities, and mutation Q146T).

Table 6 Mutations in the Cytb amino acid sequences occurring between populations of *Amblyseius swirskii*, *Kampimodromus aberrans Neoseiulus californicus* and *Typhlodromus* (*T.*) *pyri*

Position of the mutations	*A. swirskii*			*K. aberrans*				*N. californicus*						*Typhlodromus* (*T.*) *pyri*						
	Benin – Brazilian population from Israel, Idigny	Capo Verde	Reunion Island	France – Montpellier	France – Brissac	Austria – Vienna	Italy – Padova	France – Marsillargues, Midi-Pyrénées, Villeneuve-les-Maguelone	France – Villeneuve-les-Maguelone	France – Corsica	Greece	USA	Italy rearings	France – Mercantour	Austria – Vienna; Italy – Padova; Poland, Hungary, Belgium	Belgium	Hungary	Austria – Vienna	France – Restinclières	France – Valleraugue
145									Y vs M	Y vs M										
146									V vs Q	T vs Q										
161								N vs S												
178														V vs I						
180				A vs T																
181								M vs V												
184								I vs F												
185								I vs T												
201					F vs S															
205															M vs I					
215											F vs S									
216												S vs W								
222																M vs V				
223								L vs V							M vs V					
231					V vs A								T vs A							
233								V vs A												
236																		T vs I		
240														I vs M		I vs M				
254	M vs I	L vs I														K vs M				
255						I vs V										I vs V	I vs V			
256																P vs T			A vs T	P vs T
258									I vs A											
262							C vs P													
263			E vs G		G vs E				G vs E											

The mutation ‘X vs. Y’ means that the amino acid ‘X’ occurs in the populations listed, whereas the amino acid ‘Y’ occurs in the rest of the populations considered. A green background indicates a stabilizing effect of the mutation, whereas a yellow background indicates a destabilizing effect of the mutation. Red font indicates that the mutation decreases the molecule flexibility, whereas blue font indicates that the mutation increases the molecule flexibility

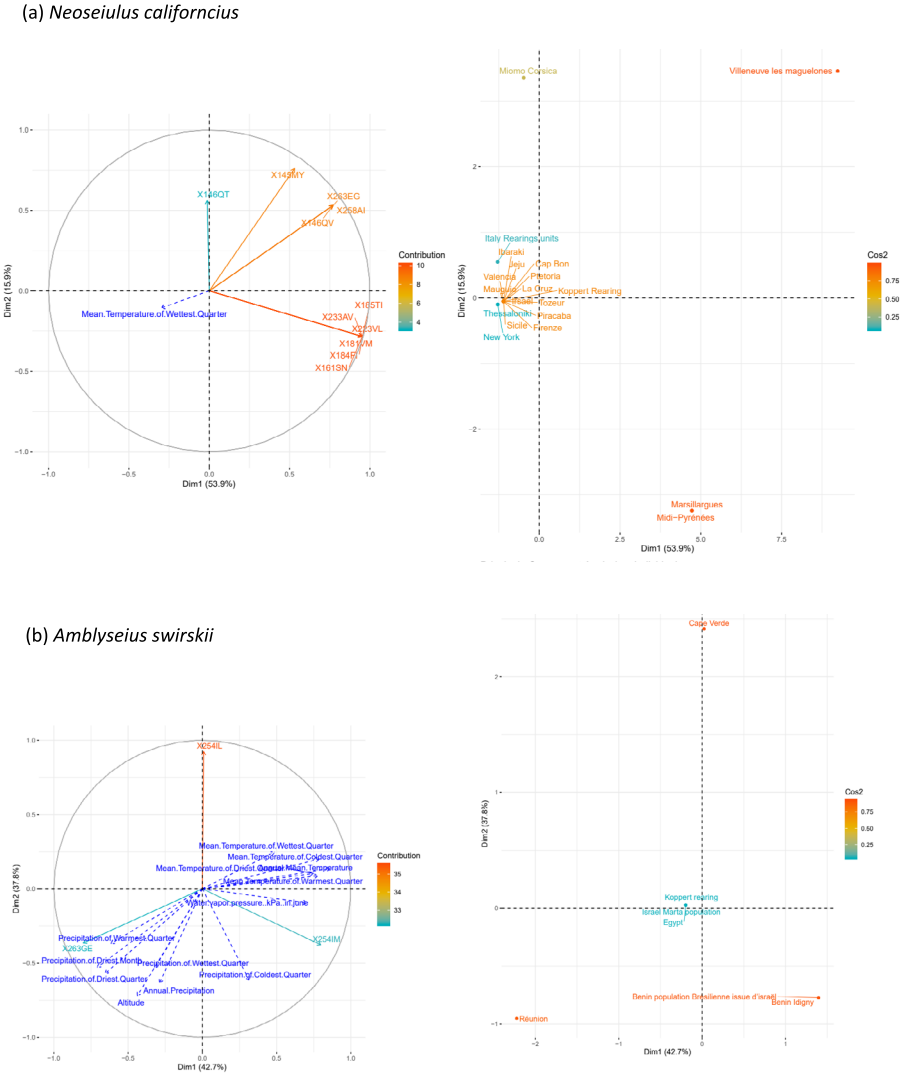


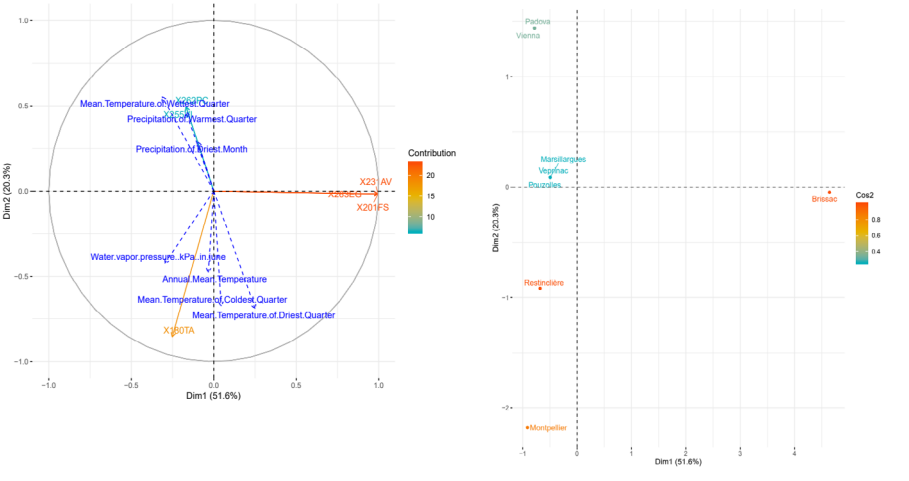
Fig. 3 Multifactorial analysis carried out on Cytb amino acid mutation pattern retrieved in different populations for each species, showing variable (mutations and climatic variables) and population representations for **a** *Neoseiulus californicus*, **b** *Amblyseius swirskii*, **c** *Kampimodromus aberrans* (with and without the Brissac population) and **d** *Typhlodromus* (*T.*) *pyri* (with and without the Belgium population). The climatic variables (additional variables) are presented in blue; the colours of both mutations (variables) and populations correspond to their more or less good representation in the system

Amblyseius swirskii

Mutations are observed between populations at two positions (three mutations) (Table 6). Mutations at position 254 separate the two Benin (I254M) and the Capo Verde (I254L)

(c) *Kampimodromus aberrans*

All populations



Without Brissac population

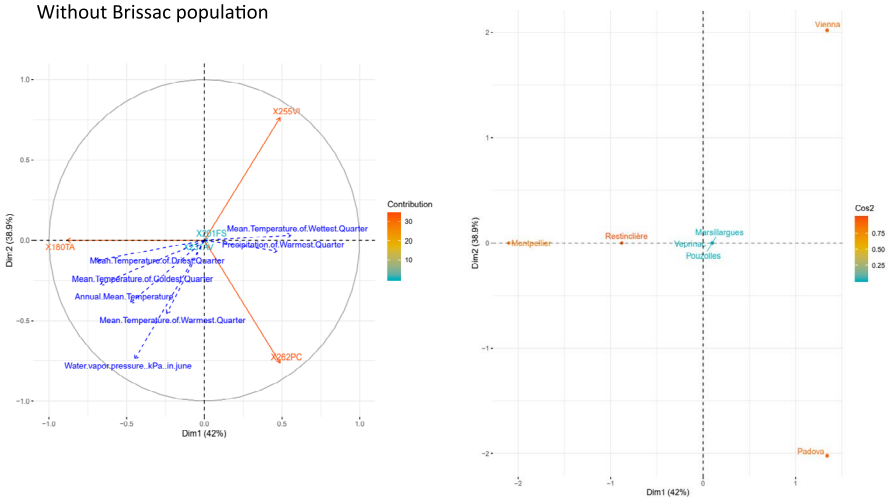
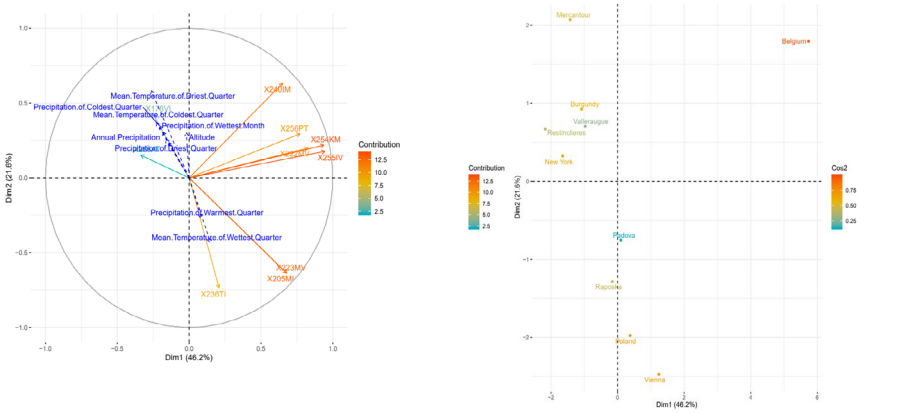


Fig. 3 (continued)

populations from the six others. The two populations from Benin have a different origin (one is issued from commercial units and one is a wild population), but because they are similar to each other and different to commercial populations, we hypothesize a mix of these two populations in the mass-rearing units and certainly a replacement of the commercial one by the wild one (Benin Idigny) (Tixier et al. 2022). Mutation G263E separates the Reunion population from the others. Mutation I254M has a destabilising effect and decreases molecule flexibility, and mutations I254L and G263E have a destabilizing effect and increase the molecule flexibility (supplementary files 1 & 2).

(d) *Typhlodromus pyri*

All populations



Without the Belgium population

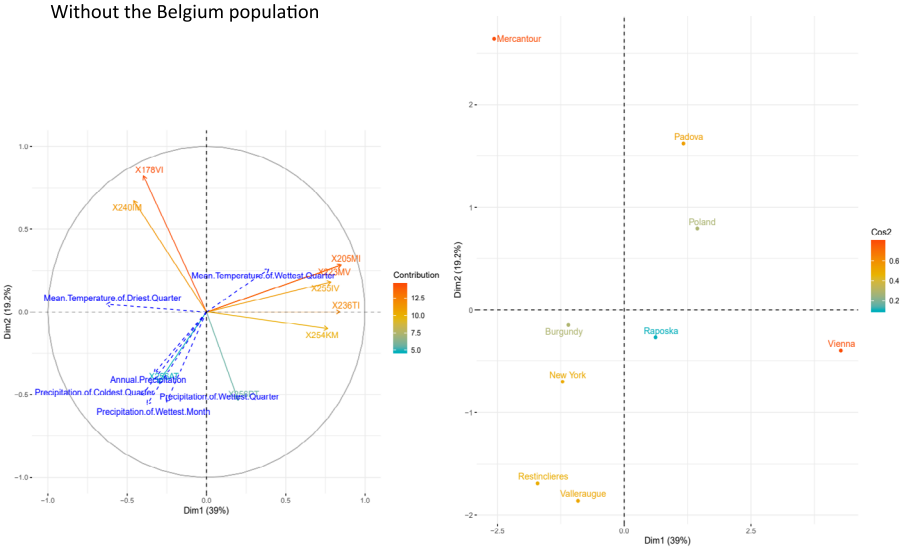


Fig. 3 (continued)

The aam pattern in the multifactorial analysis shows on axis 1 (accounting for 42.7% of the variability and mainly explained by the mutations I254M and G263E) a differentiation of the Reunion Island population from the others (Fig. 3b). This differentiation is mainly related to temperature variables. Kreiter et al. (2016) suggested that the Reunion population has been introduced in this island via commercial releases. However, it is separated from the current commercial strains, by one mutation, suggesting a probable recent evolution of this population. On axis 2 (explained by I254L), the population from Capo Verde, is well separated from the Benin and Reunion populations, the other strains having an intermediate position. The climatic factors associated to axis 2 are mainly related to

precipitation. It is interesting to note that a different mutation at the same position (I254M and I254L, from isoleucine to methionine or leucine) seems to lead to adaptation to different climatic conditions.

Kampimodromus aberrans

Six mutations are observed among the eight populations of *K. aberrans* considered (Table 6). One mutation separates a population (France-Montpellier T180A, Austria-Vienna V255I, Italy-Padova P262C) from the seven others. The population cumulating the highest number of mutations (3) is France-Brissac. No relation between plant supports and mutations is noted. All mutations have a stabilizing effect, four decrease molecule flexibility and two increase it (E263G, T180A) (Supplementary files 1 & 2).

The aam pattern in the multifactorial analysis shows a separation of the France-Brissac population from the others on axis 1 accounting for 51.6% of the variability and mainly explained by the mutations V231A, F201S and G263E (Fig. 3c). Axis 2, mainly explained by the mutation T180A, differentiates the populations from (i) Italy-Padova and Austria-Vienna, (ii) Croatia-Veprinac, France-Pouzolles, France-Marsillargues and France-Brissac, and (iii) France-Restinclières and France-Montpellier. Seven climatic variables are related to the mutation pattern, especially on axis 2; among them, five are associated to temperature. In Italy-Padova and Austria-Vienna, temperature of the wettest quarter and precipitation of the warmest quarter are higher than in France-Restinclières and France-Montpellier (and potentially related to the mutations P262C, V255I). The French population localities (Restinclières and Montpellier) are characterized by high mean temperature (annual, coldest and driest quarters), which might be related to mutation T180A. The other populations have an intermediate position and no differentiation according to climatic conditions is observed on axis 2.

Because only one specimen of Brissac was considered, an additional multifactorial analysis, excluding this specimen, was performed. The aam pattern now shows differentiation on axis 1 (explained by T180A) between the populations (i) France-Montpellier and France-Restinclières, (ii) Croatia-Veprinac, France-Pouzolles, France-Marsillargues, and (iii) Austria-Vienna and Italy-Padova. Again, the climatic features associated to temperature seem to be related to this mutation pattern. On axis 2, explained by the mutations P262C and V255I, the populations from Austria-Vienna and Italy-Padova are well separated from each other, whereas the five other populations have an intermediary position. Axis 2 is essentially related to the water pressure in June – mutations V255I (Austria-Vienna) and P262C (Italy-Padova) are negatively and positively related this variable, respectively.

Typhlodromus (*T.*) *pyri*

Ten mutations are observed between the 10 populations of *T.* (*T.*) *pyri* considered (Table 6). Two mutations (I205M, V223M) separate the populations of Austria-Vienna, Italy-Padova, Poland, Hungary and Belgium from the five others. These mutations have different properties (stabilising effect and increasing molecule flexibility for V223M and the opposite for I205M). The Belgium population is the most differentiated from the others (six mutations). Some populations share the same mutations: France-Mercantour and Belgium (M240I), Belgium and Hungary (V255I), Restinclières, France-Valleraugue and Belgium (T256P). Some mutations only prevailed in a single population: France-Mercantour (I178V),

Belgium (V222M, M254K), Austria-Vienna (I236T) and France-Restinclières (T256A). Five mutations are stabilizing and six increase molecule flexibility (supplementary files 1 & 2).

The aam pattern in the multifactorial analysis (Fig. 3d) shows three groups of populations: (i) the French and USA populations, (ii) Belgium, and (iii) Italy-Padova, Austria-Vienna, Poland and Hungary, especially on axis 1, accounting for 33.8% of the variability and mainly explained by mutations M223V, M240I, M254K, V255I and I205M. *Typhlodromus* (*T.*) *pyri* is a Palearctic species and its presence in USA is certainly due to an introduction from France, as already suggested by Tixier et al. (2012). Axis 1 is not well related to climatic features. Axis 2, mainly explained by mutations V178I, I205M, M223V, T236I and M240I, separates (i) Belgium and France-Mercantour populations, (ii) the other French and USA populations, and (iii) Italy-Padova, Austria-Vienna, Poland and Hungary. However, a gradient is observed within the latter group, with Padova and Vienna being well separated, and Hungary and Poland being intermediate. The differentiation along axis 2 is related to nine climatic features, with two main ones: mean temperature of the driest and of the wettest quarters.

As only one specimen represents the Belgium population, an additional multifactorial analysis, excluding this population, was performed. The axis shows (mainly explained by the mutations I205M, M223V, T236I, M254K, V255I) no separation in French populations and a differentiation between Padova-Hungary-Poland and Vienna populations. This differentiation seems to be associated to temperature features (mean temperature of the driest and wettest quarters). On axis 2, the France-Mercantour population is well separated from France-Restinclières and France-Valleraugue populations. The other French and USA populations have an intermediate position close to Vienna and Hungary, whereas Poland and Padova populations are intermediate between this latter group and the France-Mercantour population. This axis is mainly explained by mutations V178I and M240I, and the differentiation observed is mainly related to climate variables associated to precipitation.

Phytoseiulus persimilis

No mutation separates the eight populations considered.

Cytb amino acid variation within populations

Neoseiulus californicus

Mutations are observed at 13 sites at the intra-population level, but only in five populations among the 20 considered, showing a low intra-population variation concerning only a few specimens (Table 7). The highest number of mutations is observed for the population Italy-rearing (eight positions). Among the 13 mutations, 12 are observed in only one population and one is observed in two populations (position 264 for France-Corsica and France-Mauguio). Of the five populations showing mutations, three are ‘commercial’ strains.

Kampimodromus aberrans

Twelve sites show mutations at the intra-population level for five populations among the eight considered. The highest number of mutations is observed for France-Restinclières

Table 7 Positions and numbers of mutations in amino acid sequences of the Cytb fragment considered within the populations of *Amblyseius swirskii*, *Kampimodromsus aberrans*, *Neoseiulus californicus*, *Phytoseiulus persimilis* and *Typhlodromus* (*T.*) *pyri*

Position of the mutations	*A. swirskii*					*K. aberrans*					*N. californicus*					*P. persimilis*		*T.* (*T.*) *pyri*							
	Egypt	Reunion Island	Benin – Brazilian population from Israel	Benin – Idigny	Capo Verde	Italy Padova	France – Restinclières	France – Montpellier	France – Pouzolles	France – Marsillargues	Italy Rearings	Brazil	Tunisia – Cap Bon	France – Corsica	France – Mauguio	Italy	Tunisia, *Amaranthus reflexus*	France – Burgundy	Italy – Padova	Poland	Hungary	France – Mercantour	France – Restinclières	Austria – Vienna	France – Vallerague
143																		x	x						
146											x														
159						x																			
160						x																			
175																x									
178																									
180							x																		
191																			x						
202												x													
204	x																								
210																	x								
218											x														
222																		x	x	x					
225		x																							
226	x																								
227			x																						
228				x																					
233																									
236																				x	x				
237								x																	

Table 7 (continued)

Position of the mutations	*A. swirskii*					*K. aberrans*					*N. californicus*					*P. persimilis*		*T. (T.) pyri*							
	Egypt	Reunion Island	Benin – Brazilian population from Israel	Benin – Idigny	Capo Verde	Italy Padova	France – Restinclières	France – Montpellier	France – Pouzolles	France – Marsillargues	Italy Rearings	Brazil	Tunisia – Cap Bon	France – Corsica	France – Mauguio	Italy	Tunisia, *Amaranthus reflexus*	France – Burgundy	Italy – Padova	Poland	Hungary	France – Mercantour	France – Restinclières	Austria – Vienna	France – Vallerauge
238							x	x																	
239				x	x														x						
240									x													x			
241				x							x														
244							x		x																
246											x														
248													x												
249							x																x		
250							x	x																	
251											x														
254					x																			x	
255											x								x					x	
256																		x			x			x	x
257		x																							
258											x														
260							x			x															
261											x														
262														x											
263										x										x			x		
264										x				x	x			x			x				
N° mutations	2	2	1	3	2	2	6	3	2	3	8	1	1	2	1	1	1	3	4	3	3	1	2	3	1

(six positions) (Table 7). Among the 12 variable positions, eight are variable in only one population and four in two populations.

Typhlodromus (T.) pyri

Twelve sites show mutations at intra-population level in eight populations among the 10 considered (Table 7). The highest number of mutations is observed for Italy-Padova (five sites), France-Burgundy (four sites), Poland, Hungary and Austria-Vienna (three sites). Among the 12 sites, five are variable in only one population, five in two populations, one (site 222) in three populations, and one (site 256) in four populations.

Amblyseius swirskii

Nine sites show mutations at the intra-population level in five populations among the nine considered (Table 7). The highest number of mutations is observed for Benin-Idigny (three positions). Among the nine sites, eight are variable in one population and two in two populations (Capo Verde, Benin-Idigny).

Phytoseiulus persimilis

A low number of mutations is observed at the intra-population level, with two mutations recorded in two populations (Italia and Tunisia) (Table 7).

Conclusive remarks on aam patterns in the Cytb fragment

Mutations between populations were observed in four species among the five considered, and for all species mutations within populations were recorded. The number of mutations was higher at intra-population (42 positions) than at inter-population level (25 positions). Even if 19 mutations are globally retrieved at intra- and inter-population levels, the number of common mutations at intra- and inter-population levels for a single species is low: three for *N. californicus*, two for *K. aberrans*, seven for *T.* (*T.*) *pyri* and one for *A. swirskii*. One possible explanation for high variability within a population might be a mix with other populations and/or adaptations to particular biotic and abiotic constraints. On the 25 variable sites at the inter-population level, mutations at the same site are observed in two species—site 223 for *T.* (*T.*) *pyri* and *N. californicus*, site 231 for *N. californicus* and *K. aberrans*, site 254 for *T.* (*T.*) *pyri* and *A. swirskii*, site 255 for *T.* (*T.*) *pyri* and *K. aberrans*—and three species (site 263 for *K. aberrans*, *N. californicus* and *A. swirskii*). On the 42 sites variable at intra-population level, seven and one mutations are retrieved in two and three species, respectively.

Those results confirm the high mutation rate of the Cytb amino acid sequence and also its robustness, as already stated for insects by Simmons and Weller (2001). No relation between plant supports and mutations was observed. No relation between mutations and taxonomy was noted either, suggesting polyphyletic and/or recent adaptations. Amino acid variability is globally lower in 'commercial' species (*N. californicus*, *A. swirskii* and *P. persimilis*) than in 'wild' ones (*T.* (*T.*) *pyri* and *K. aberrans*). 'Commercial' populations are differentiated from the 'wild' ones by several mutations; however, we do not have enough information on the biological features of these populations to conclude on the

effects of such mutations on the protein functions and their consequences on population performances.

As in previous studies on *T.* (*T.*) *phialatus*, *T.* (*A.*) *recki*, *T.* (*A.*) *rhenanoides* and *P. macropilis* (Tixier et al. 2021; Queiroz et al. 2021), mutations according to localities are also observed here. For 'wild' species, but also for some 'wild' populations of the commercial species, relations between mutation patterns and climatic features were observed. We did not detect mutations clearly related to a single climatic feature, suggesting that (i) climatic factors (at least those herein considered) can have combined effects, and (ii) other factors, not herein considered, can act on mutation selection. However, globally, the main factors associated with aam pattern seem to be temperature (especially temperature of the wettest quarter) and, to a lesser extend, precipitation. A same mutation (V255I) is retrieved in *K. aberrans* (Austria) and *T.* (*T.*) *pyri* (Poland, Hungary) populations, in relation to climate features. Climatic effects on protein function are not well documented, especially in insects. As arthropod's internal temperature is directly related to external temperature, we can assume that external temperatures could lead to adaptation in proteins, as highly temperature sensitive components (Somero 2010).

Studies on *Lottia* and anchovies have shown that a single amino acid substitution in a protein (dehydrogenase enzymes for *Lottia* and COI for anchovies) can affect its stability and its function, and can explain adaptation to temperature (Dong and Somero 2009; Silva et al. 2014). Somero (2010) stated that a minor number of sequence changes is required for temperature adaptation, and that many sites in a protein are able to support adaptive change to climatic conditions. In woolly mammoth, mutations in several proteins, including Cytb and COI, could be related to cold conditions and oxygen availability (Ngatia et al. 2019). Cytb expression in mosquitoes was affected by environmental conditions, including temperature (Zhao et al. 2009). To our knowledge, the present study is the first one attempting to relate mutation occurrence to a large number of climatic variables. Even if, globally, the present results seem to confirm an effect of climatic conditions on the Cytb protein in Phytoseiidae mites, we are not able to determine how such adaptations to climatic conditions would modify protein functions. To answer this question, experiments focusing on protein activity in different populations and at multiple temperatures would be required.

Mutations on the Cytb amino acid sequence have been related to resistance to bifenazate, acequinocyl and Qo inhibitors in *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). These mutations were not retrieved in the 234 specimens considered, except at site 262 in the population of Italy-Padova and in some specimens of *N. californicus* from France-Corsica and Poland. For all these specimens, however, the mutation is different from that in Tetranychidae (P262T for Tetranychidae vs. P262C in Phytoseiidae). As we do not have information on the potential resistance of the Phytoseiidae specimens bearing this mutation, we cannot determine whether the mutation P262C observed here might be associated to resistance to those pesticides.

COI amino acid variation between populations within species

Typhlodromus (*T.*) *pyri*

Four mutations were observed among the eight populations of *T.* (*T.*) *pyri* considered (Table 8). Three mutations separate one population from the others (Hungary: I162V, Austria-Vienna: T183A, Poland: L241T). Mutation M182L separates the populations of

Table 8 Mutations in the COI amino acid sequences occurring between populations of *Amblyseius swirskii*, *Phytoseiulus persimilis*, *Phytoseius finitimus* and *Typhlodromus* (*T.*) *pyri*

Position of the mutations	*A. swirskii*		*Phytoseiulus persimilis*			*Phytoseius finitimus*	*T. (T.) pyri*			
	Syngenta	Syngenta, Koppert	Tunisia - Cap Bon	France - Montpellier	Spain - Valencia	Italy – Padova, *Viburnum tinus*	Hungary - Raposka	Austria - Vienna	Poland	France - Burgundy, valraugue; USA
28						L vs A				
67						S vs A				
92						I vs L				
94						V vs I				
95						N vs H				
96						S vs A				
120						M vs L				
135						I vs V				
143						I vs T				
148						A vs T				
162							V vs I			
182										L vs M
183								A vs T		
195						V vs I				
241									T vs L	
247		T vs H	T vs H	T vs H						
248	Q vs P				P vs Q					
249				K vs E						

The mutation ‘X vs. Y’ means that the amino acid ‘X’ occurs in the populations listed, whereas the amino acid ‘Y’ occurs in the rest of the populations considered. A green background indicates a stabilizing effect of the mutation, whereas a yellow background indicates a destabilizing effect of the mutation. Red font indicates that the mutation decreases the molecule flexibility, whereas blue font indicates that the mutation increases the molecule flexibility

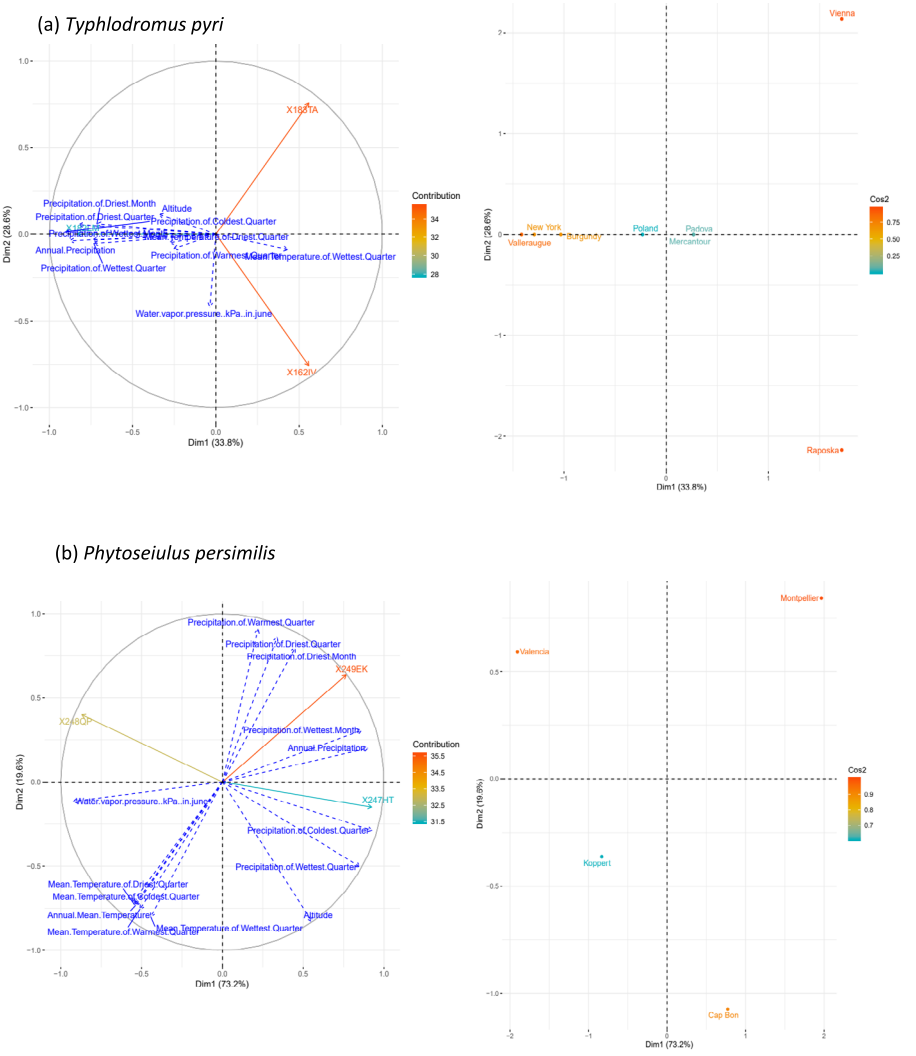


Fig. 4 Multifactorial analysis carried out on Cytb amino acid mutation pattern retrieved in different populations considered for each species, showing variables (mutations and climatic variables) and population representations for (**a**) *Typhlodromus* (*T.*) *pyri*, (**b**) *Phytoseiulus persimilis* and (**c**) *Phytoseius finitimus.* The climatic variables (additional variables) are presented in blue; the colours of both mutations (variables) and populations correspond to their more or less good representation in the system

France-Burgundy, France-Valleraugue and USA from the others. As also suggested by the Cytb results, the population of *T.* (*T.*) *pyri* introduced in USA seems to have a French origin. The four mutations have a destabilizing effect and increase the molecule flexibility (supplementary files 1 & 2).

The aam pattern in the multifactorial analysis (Fig. 4a) shows a separation of three groups on axis 1 accounting for 33.1% of the variation and mainly explained by mutations M182L, T183A and I162V: (i) France-Valleraugue, France-Burgundy, USA, (ii) France-Mercantour, Poland, Italy-Padova, and (iii) Austria-Vienna and Hungary. Many climatic

(c) *Phytoseius finitimus*

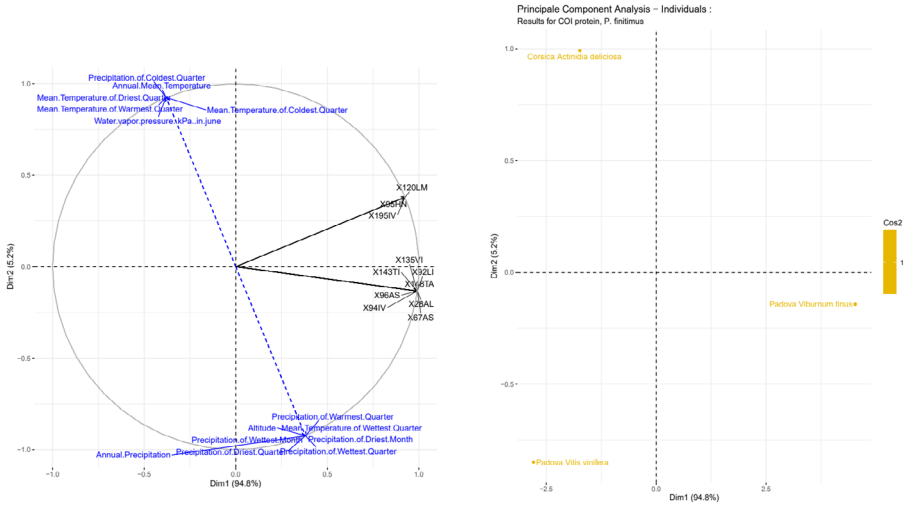


Fig. 4 (continued)

variables (mainly precipitation variables) are related to axis 1. Axis 2 separates the Austria-Vienna and Hungary populations, the six others having an intermediate position. No climatic feature was well related to axis 2 (mainly explained by T183A and I162V).

Amblyseius swirskii

Two mutations (at positions H247T and P248Q) separate the Syngenta population from the seven others (Table 8). The mutation H247T was common between Koppert and Syngenta, and separates these populations from the others. All other populations, whatever their locality, host plant, or whether they are 'wild' or 'commercial', have the same amino acid sequence.

Phytoseiulus persimilis

Three mutations were observed among the four populations considered (Table 8). Mutation H247T separates France-Montpellier and Tunisia-Cap Bon from the other populations, two mutations (H247T, E249K) separate France-Montpellier from the others, and one mutation (Q248P) separates the Spain-Valencia population from the others. Mutation H247T has a destabilising effect and decreases molecule flexibility, mutation Q248P has a destabilising effect and increases molecule flexibility, and mutation E249K has a stabilising effect and increases molecule flexibility (Supplementary files 1 & 2).

The aam pattern in the multifactorial analysis (Fig. 4b) shows that the four populations are well separated from each other. Among the numerous climatic variables well represented in the system, most related to the three mutations are precipitation and water vapour pressure. However, caution should be paid, as the number of populations and mutations is low.

Typhlodromus (*A.*) *recki*

No mutation prevailed in a population or group of populations. This can be due to the low number of populations (two) considered.

Phytoseius finitimus

Eleven mutations were observed among the three populations considered. All these mutations separate one population (Padova-*Viburnum tinus*) from the others (Table 8). Four mutations have a stabilising effect, seven have a destabilizing effect, three increase the molecule flexibility, whereas eight decrease it (Supplementary files 1 & 2). As the three populations were collected on different plants, plant support does not seem to affect the mutation pattern observed. The Italy-Padova *V. tinus* population is geographically closer to Italy-Padova *Vitis vinifera* than this latter population is from France-Corsica *Actinidia deliciosa*. Thus, geographical distance cannot explain the mutation pattern either. The multifactorial analysis shows that none of the climatic features considered are clearly related to the aam pattern (Fig. 4c). The two similar populations are collected in crops whereas the Italy-Padova *V. tinus* population is collected in uncultivated areas. Effects of agricultural practices, such as pesticide applications, might explain the different amino acid pattern. However, to our knowledge, no previous study has shown such an effect on COI amino acid composition.

COI amino acid variation within populations

Typhlodromus (*T.*) *pyri*

Six sites show mutations at intra-population level, for two populations among the eight considered (Table 9). The highest number of mutations was observed for France-Valleraugue (five positions). Among these six sites, two show mutations at inter-population level (sites 182 and 241). The France-Valleraugue population is reared under laboratory conditions. Each year, new specimens are collected on *Rubus* sp. at Valleraugue and added to the lab colony. This population renewing could explain the high number of mutations at intra-population level (population mix).

Amblyseius swirskii

Intra-population mutations were observed at eight positions for three populations among the eight considered (Table 9). The highest number of mutations was observed in the 'wild' Capo Verde population (six positions). One mutation recorded at inter-population level also occurs at the intra-population level (position 248).

Typhlodromus (*A.*) *recki*

Intra-population mutations are observed at eight sites for the two populations (Table 9). The highest number of mutations is observed in the Palermo population (seven positions).

Table 9 Positions and numbers of mutations in amino acid sequences of the COI fragment considered within the populations of *Amblyseius swirskii*, *Phytoseiulus persimilis*, *Phytoseius finitimus*, *Typhlodromus* (*Anthoseius*) *recki* and *Typhlodromus* (*T.*) *pyri*

Position of the mutations	*A. swirskii*			*P. persimilis*	*P. finitimus*			*T.* (*A.*) *recki*		*T.* (*T.*) *pyri*	
	Capo Verde	France-Réunion	Koppert	Koppert	Italy—Padova, *Viburnum tinus*	France—Corsica, *Actinidia deliciosa*	Italy—Padova, *Vitis vinifera*	Italy—Palermo	France—Montferrier-sur-Lez	France—Vallerau-gue	France -Bur-gundy
51	x										
67					x						
77								x			
90								x			
92					x						
94					x						
95					x	x					
96					x						
100								x			
103								x			
120					x	x					
135	x				x					x	
143					x						
147								x			
148					x						
162					x						
182						x					x
186								x	x		
195					x	x				x	
200	x										
201						x					

Table 9 (continued)

Position of the mutations	*A. swirskii*			*P. persimilis*	*P. finitimus*			*T.* (*A.*) *recki*		*T.* (*T.*) *pyri*	
	Capo Verde	France-Réunion	Koppert	Koppert	Italy—Padova, *Viburnum tinus*	France—Corsica, *Actinidia deliciosa*	Italy—Padova, *Vitis vinifera*	Italy—Palermo	France—Montferrier-sur-Lez	France—Valleraugue	France-Burgundy
202						x					
208								x			
225							x			x	
227	x										
239		x									
241										x	
242										x	
243	x										
244	x										
247									x		
248			x	x			x				
249				x	x						
250				x	x						
251				x							
N° mutations	6	1	1	4	13	6	2	7	2	5	1

Phytoseius finitimus

Eighteen sites show mutations at intra-population levels, for the three populations considered (Table 9). The highest number of mutations is observed for Padova *V. tinus* (13 sites), followed by Corsica *A. deliciosa* (six sites) and Padova *V. vinifera* (two sites). Nine of these mutations are also observed at inter-population level. It is worth to note that the high intra-population variation observed for Italy-Padova *V. tinus* is due to one specimen, whose amino acid composition is globally the same as that of the specimens of the two other populations. This can be due to dispersal (mix of populations).

Phytoseiulus persimilis

Four mutations were observed in only one population (commercial strain Koppert) (Table 9). Two sites (248, 249) also concerned mutations at inter-population level.

Conclusive remarks on aam patterns in the COI fragment

As for Cytb analysis, the mutation rates are higher at intra-population (35 positions) than at inter-population levels (18 positions). Sixteen mutations are observed both at inter- and intra-population level, but – as for the Cytb fragment – not within the same species: three for *T.* (*T.*) *pyri*, one for *A. swirskii*, 10 for *P. finitimus* and three for *P. persimilis*. On the 18 positions variable at the inter-population level, mutations at the same site are observed only in two species (*A. swirskii* and *P. persimilis*, for positions 247 and 248). On the 35 sites variable at the intraspecific level, five carry mutations for two species, and two for three species (positions 135 and 248). As for Cytb, it seems that mutations are not fixed in the same way for intra- and inter-population levels, and no similarity between muted sites is observed across the two sub-families considered. High mutation rates were observed for the COI amino acid sequence, but those values are lower than for Cytb, suggesting that COI is less robust to changes than the Cytb sequence, as already stated for insects by Simmons and Weller (2001).

Some mutations were observed in COI acid amino sequences, prevailing in a population or a group of populations. As for the Cytb amino acid sequences, neither plant support nor taxonomical characteristics seem to be associated to the mutation patterns. Similar to the Cytb results, lower variation was observed in 'commercial' than in 'wild' species. Finally, for the species both analysed with COI and Cytb markers, the relation between aam pattern and climatic features is not similar, suggesting that these two proteins answer in a different way to external constraints and might be markers of different adaptations, as already observed for *T.* (*A.*) *recki* for the 12S and Cytb markers (Tixier et al. 2021). The COI mutations herein observed are mostly associated to precipitation features. This is in accordance with results obtained on anchovy, where COI mutations not seem to be selected according to temperature, contrary to Cytb (Pappalardo et al. 2015). However, the number of species, populations and mutations considered is low and this conclusion should be corroborated by further comparisons. Moreover, for *P. finitimus* such an effect was not shown. Literature on effects of precipitation (and humidity conditions) on mutation selection is quite scarce, especially for arthropods. In flies, mutations in proteins (ionotropic receptors) affect their sensitivity to dry environments (Enjin et al. 2016). The functional effects of COI mutations are not well investigated in arthropods, whereas functional impacts have been reported on hummingbird flight (Dunn et al. 2019) and human fecundity and disease development

(Zhen et al. 2015). Phytoseiidae mites are affected by moisture and most species are susceptible to dry conditions (limiting egg survival) (Rowles et al. 2009; Ferrero et al. 2010). Adaptations to moisture conditions would not be surprising for such organisms.

General conclusion

This study shows mutations in COI and Cytb amino acid sequences and investigates for the first time factors affecting these mutations in Phytoseiidae mites. Despite the important functions of these two proteins involved in the respiratory chain, many mutations were observed both at inter- and intra-population levels, indicating their robustness. Globally, purifying selection was observed confirming results of previous studies on arthropods (Sabir et al. 2019). The present study also shows that mutations in the Cytb and COI amino sequences are related to localities, confirming similar trends observed in preliminary studies carried out on the Cytb protein. Mutation patterns seem to be related to climatic conditions, especially to temperature and precipitation for the Cytb fragment, and mainly precipitation and moisture for the COI fragment. However, no unique climatic factor was clearly related to the aam pattern, suggesting that a mix of climatic factors could explain adaptation and/or that the climatic factors investigated should be refined. This work clearly opens new research lines on proteomic approaches and impacts on life traits, in line with the perspectives addressed by Hoffman and Willi (2008). We did not have information on biological traits of the populations considered to conclude on effects of mutations on their performances. Clearly, the present results constitute the basis of further innovative studies, to determine how aam pattern could be used as proxy of (i) adaptations to climatic conditions, and (ii) biological performances for improving the efficiency of predatory populations, both in a context of climate change.

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Author contributions MT supervised the studies, wrote the manuscript and carried out statistical analyses. LT compiled the data sets, and carried out statistical analyses.MD carried out the molecular experiments.

Data availability Tixier M.-S. 2020. DNA sequences of COI and CytB. Available freely in GenBank.

Declarations

Conflict of interest The authors declare no competing interests.

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