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Investigating host associations of the European Red Mite *Panonychus ulmi* (Acari: Tetranychidae) through an integrative approach

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Abstract
Panonychus ulmi (Koch) (Acari: Tetranychidae), the European red mite (ERM), is a polyphagous spider mite that infests economically important fruit crops, such as apple trees and grapevines. This study investigated potential morphological, reproductive, and genetic differences among populations associated with these hosts. We performed (i) morphological characterization of females from 15 sampling areas; (ii) molecular characterization of 19 populations from six different host species and six countries, using two fragments of the cytochrome c oxidase subunit I (*COI*) gene and the internal transcribed spacers ITS1 and ITS2; and (iii) reproductive compatibility tests between apple and grapevine-associated populations. Dorsal setae showed no consistent morphological differences. In contrast, genital shield setae differed significantly, with higher values in apple-associated populations. *COI* and ITS analyses identified a genetic lineage (Group I and Group I–ITS) capable of colonizing both hosts. However, combined phylogenetic analysis revealed a subclade composed exclusively of grapevine populations. Crosses indicated that the female's host of origin influenced reproductive performance, with higher oviposition by females originating from apple. Overall, the results do not support distinct *P. ulmi* lineages specifically associated with the two evaluated hosts. Low intraspecific divergence is consistent with a single colonizing species that expanded its niche from apple trees to grapevines without undergoing genetic structuring. A distinct lineage (Group II) present in Chile, Canada, and Russia suggests the possible circulation of a genotype that may be taxonomically confused with *P. ulmi*, warranting further taxonomic investigation and diagnostic vigilance.

Keywords: Integrative taxonomy, biotypes, reproductive compatibility, morphological traits, phylogeny

Introduction

Panonychus ulmi (Koch) (Acari: Tetranychidae), known as the European red mite (ERM), is a polyphagous spider mite reported on more than 170 host plants, including numerous economically important fruit crops (Migeon & Dorkeld, 2026; Kasap & Atlihan, 2021; Joshi et al., 2023). It is widely distributed throughout temperate fruit-growing regions of Europe, Asia, North and South America, Oceania, and parts of Africa (Migeon & Dorkeld, 2026), where it is primarily recognized as an important pest of apple orchards.

Although apple remains the principal host of ERM, the mite has also been reported on grapevines in several producing regions. In European vineyards, ERM is generally considered a secondary pest, with outbreaks often associated with disruptions of biological control (Duso et al., 2012; Zanettin et al., 2021). In contrast, damaging populations have been reported in South American vineyards, demonstrating that grapevines can support the establishment and population growth of ERM (Ferla & Botton, 2008; Johann et al., 2019).

In Brazil, ERM was first recorded during the interception of apples imported from Argentina (Flechtmann, 1967) and was subsequently reported in commercial apple orchards in Santa Catarina, Brazil's largest apple-producing state (Bleicher, 1974; Ferreira, 1997), followed by records from other states in southern Brazil. More than three decades later, ERM was first reported infesting grapevines (*Vitis vinifera* L., cultivar Merlot) in Rio Grande do Sul (RS), Brazil's largest grape-producing state and second-largest apple producer, where apple orchards and vineyards frequently occur in proximity (Abrafrutas, 2024; Ferla & Botton, 2008).

Interactions between phytophagous mites and their host plants are major drivers of host-associated differentiation and diversification (Drès & Mallet, 2002; Van Leeuwen & Dermauw, 2016; Guzman-Valencia et al., 2017; Villacis-Perez et al., 2021). Accordingly, host-associated lineages in mites can be investigated using an integrative approach that combines morphological, molecular, ecological, and reproductive evidence (Skoracka et al., 2015).

Molecular markers such as mitochondrial cytochrome c oxidase subunit I (COI) and the nuclear internal transcribed spacer (ITS) are widely used to investigate phylogenetic relationships and host-associated differentiation (Knee et al., 2012; Mendonça et al., 2025), while reproductive compatibility studies provide complementary evidence of host adaptation, gene flow, and reproductive isolation among populations (Villacis-Perez et al., 2021).

The proximity between apple orchards and vineyards in southern Brazil raises questions about the relationships between ERM populations associated with these crops. Whether grapevine populations represent host-associated lineages resulting from adaptation to a new host or remaining part of a single reproductively compatible population is currently unknown. To address this knowledge gap, we adopted an integrative approach combining morphometric, reproductive compatibility, and molecular analyses to investigate the relationships between ERM populations associated with apple and grapevine.

Material and Methods

Sampling

A total of 23 ERM populations were studied: 10 populations from apple trees (nine from *Malus domestica* Borkh. and one from *Malus pumila* Mill.), 11 populations from grapevines (ten from *V. vinifera* and one from *Vitis labrusca* L.), one population from almond (*Prunus amygdalus* Batsch), and one from pear (*Pyrus communis*

L.) (Table 1), from six countries (Brazil – BRA; Chile – CHL; Japan – JPN; Morocco – MAR; South Africa – ZAF; and the United States of America – USA) (Figure 1), collected during the 2012–13 and 2014–15 seasons.

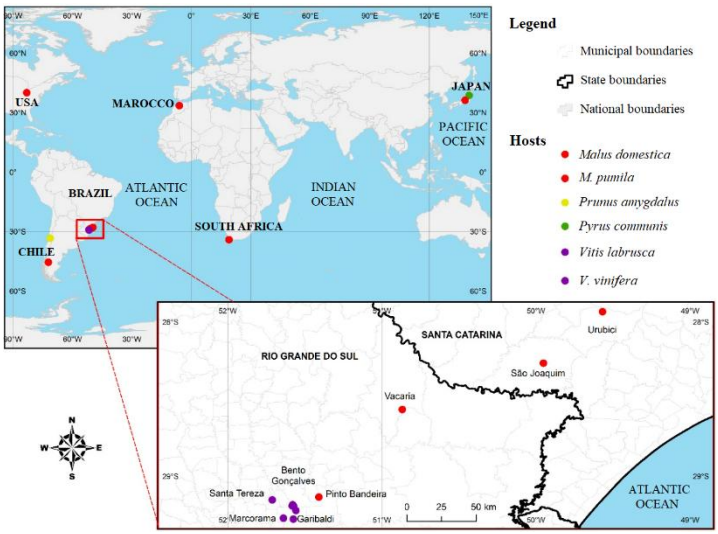


Fig. 1 Geographic distribution of *Panonychus ulmi* (ERM) populations included in this study and their respective host plants

Morphological analyses

ERM specimens from five populations collected from apple trees (*M. domestica*) and 10 from grapevines (nine from *V. vinifera* and one from *V. labrusca*) were used (Table 1), from Brazil (14) and the United States of America (USA) (one). Ten females from each population were slide-mounted in dorsoventral position in Hoyer's medium, following the method described by Jeppson (1975).

Morphological analyses were conducted at the Laboratory of Acarology, University of Vale do Taquari - Univates, Lajeado, RS, Brazil, by examining taxonomically relevant characters for Tetranychidae systematics (Baker & Tuttle, 1994). All measurements (µm) were taken with a Leica DMLS phase-contrast optical microscope (Germany) equipped with a calibrated ocular micrometer at 40× magnification.

Morphological characters evaluated included length and width of the dorsal shield; length of dorsal setae (*v2, sc1, sc2, c1, c2, c3, d1, d2, e1, e2, f1, f2,* and *h1*); inter-setal distances on the dorsal shield (*v2–v2, sc1–sc1, sc2–sc2, c1–c1, d1–d1, e1–e1, f1–f1,* and *h1–h1*); length of genital shield setae (*ag, g1, g2, ps1, ps2, h2, and h3*); inter-setal distances on the genital shield (*ag–ag, g1–g1, g2–g2, ps1–ps1, ps2–ps2, h2–h2, and h3–h3*); length of leg segments (coxa, trochanter, femur, genu, tibia, and tarsus); setae of legs I–IV; distance from the base of tarsus I to the base of the duplex setae; and length and width of the stylophore.

Statistical analysis was performed using Jamovi version 2.4 (2022) and R (R Core Team, 2022). Intercorrelations among quantitative variables of measured setae were analyzed using the Principal Component Analysis (PCA), employing Varimax rotation and parallel analysis for component extraction. Model quality was assessed using Bartlett's test of sphericity, which yielded a significant value ($\chi^2 = 1{,}338$; df = 190; $p < 0.001$), and the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy, which was 0.88, indicating excellent suitability. To test whether the independent variable "Host" influenced PCA-derived components, Generalized Linear Models (GLzM) with normal distribution were fitted. Model selection was based on the lowest Akaike Information Criterion (AIC) value. Bonferroni correction was applied for post hoc comparisons. A significance level of $p < 0.05$ was adopted for all analyses, and estimates are presented with 95% confidence intervals (CIs).

Table 1 Collection data, host plants, and localities of the *Panonychus ulmi* (ERM) populations included in this study.

* = Morphometric analyses; ■ = Molecular characterization; ▲= Reproductive compatibility

No.	IDs	Host	Collection		
			Locality	Date	Geographic coordinates
1	Pu2■*	*Vitis vinifera* – Merlot	Santa Tereza – RS – BR	01/29/2013	29°09'14.3"S 51°42'38.3"W
2	Pu3*	*V. vinifera* – Table grape	Bento Gonçalves – RS – BR	01/29/2013	29°11'38.4"S 51°34'03.2"W
3	Pu4■*	*V. vinifera* – Trebbiano	Bento Gonçalves – RS – BR	02/06/2013	29°11'07.9"S 51°34'40.2"W
4	Pu5*	*V. vinifera* – Merlot	Monte Belo – RS – BR	02/06/2013	29°10'33.0"S 51°37'22.2"W
5	Pu6■*	*V. vinifera* – Merlot	Bento Gonçalves – RS – BR	02/06/2013	29°11'35.0"S 51°34'53.3"W
6	Pu7*	*V. vinifera* – Merlot	Bento Gonçalves – RS – BR	02/06/2013	29°13'23.2"S 51°33'28.6"W
7	Pu8*	*V. vinifera* – Cabernet Sauvignon	Bento Gonçalves – RS – BR	02/06/2013	29°11'46.3"S 51°34'53.3"W
8	Pu9■*	*V. vinifera* – Pinot Noir	Bento Gonçalves – RS – BR	02/06/2013	29°13'22.9"S 51°33'26.5"W
9	Pu11■*	*V. vinifera* – Trebbiano	Marcorama – RS – BR	02/15/2013	29°16'23.4"S 51°38'17.4"W
10	Pu21■▲	*V. vinifera* – Merlot	Bento Gonçalves – RS – BR	11/26/2014	29°11'32.6"S 51°34'22.2"W
11	Pu25.1■*	*V. labrusca*	Garibaldi – RS – BR	03/04/2015	29°16'44.0"S 51°34'25.1"W
12	Pu20■	*Prunus amygdalus*	Curacaví – Santiago – Chile	03/01/2013	33°23'54.3"S 71°06'53.5"W
13	Pu23■	*Pyrus communis*	Yamagata – Japan	06/11/2014	38°37'28.3"N 140°10'52.1"E
14	Pu10■*	*Malus domestica* – Fuji	Pinto Bandeira – RS – BR	02/07/2013	29°08'10.2"S 51°24'30.6"W
15	Pu12■*	*M. domestica* – Gala	Urubici – SC – BR	02/18/2013	27°56'01.3"S 49°34'4.17"W
16	Pu13■*	*M. domestica* – Gala	São Joaquim – SC – BR	02/18/2013	28°16'02.5"S 49°57'05.6"W
17	Pu19.1■*	*M. domestica*	Columbus – Ohio – USA	07/02/2013	39°57'55.6"N 82°59'46.1"W
18	Pu22.2■▲*	*M. domestica* – Gala	Vacaria – RS – BR	12/17/2014	28°34'5.40"S 50°52'1.53"W
19	Pu24■	*M. pumila*	Nagano – Japan	06/10/2014	36°09'55.8"N 138°05'55.0"E
20	Pu26■	*M. domestica* – Gala	Oulmes – Morocco	07/05/2014	33°25'30.6"N 6°00'06.0"W
21	Pu28■	*M. domestica*	Coyhaique – Chile	02/04/2013	45°34'15.6"S 72°04'02.8"W
22	Pu29■	*M. domestica*	Elgin – Western Cape – South Africa	02/12/2013	34°08'44"S 19°02'35"E
23	Pu30■	*M. domestica*	Western Cape – Elgin – South Africa	02/12/2013	19° 3′10″E 34° 9′10″S

BR: Brazil; RS: Rio Grande do Sul; SC: Santa Catarina

Molecular characterization

Ten ERM females per population were used from 19 populations: 10 from apple trees (nine from *M. domestica* and one from *M. pumila*), seven from grapevines (six from *V. vinifera* and one from *V. labrusca*), one from pear (*P. communis*), and one from almond (*P. amygdalus*) (Table 1), from Brazil (11), Chile (two), Japan (two), South Africa (two), USA (one), and Morocco (one). Collected mites were stored in absolute ethanol at −20°C.

DNA extraction

Total DNA extraction was performed using a nondestructive method with the DNeasy Blood & Tissue Kit (QIAGEN®, USA), following the protocol adapted from Dowling et al. (2011) and Mendonça (2011). After extraction, specimens were recovered as vouchers, mounted on slides in Hoyer's medium, and deposited in the collection of the Plant Germplasm Quarantine Laboratory, Embrapa Genetic Resources and Biotechnology (Cenargen), Brasília, Federal District (DF), Brazil.

Four DNA fragments were amplified by PCR for each individual. Two fragments of the mitochondrial COI gene were amplified: the COI barcoding region, amplified with primers LCO1490 and HCO2198 (Folmer et al., 1994), and the region here referred to as COI Navajas, amplified with primers C1J1718 and 773 (Simon et al., 1994; Navajas et al., 1994). Two regions of the nuclear ribosomal DNA were also amplified: internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), with primers located in the 18S, 5.8S, and 28S genes (Table S1).

Amplification and sequencing of fragments

All PCR reactions were performed in a final volume of 25 µL containing 2 µL of DNA at an average concentration of 5.9 ng/µL, using a *Taq* DNA Polymerase kit (Qiagen®, Hilden, Germany). Primers, reagents, and cycling parameters for each fragment are detailed in Tables S1 and S2.

PCR products were analyzed by electrophoresis on 1% agarose gels stained with GelRed® Nucleic Acid Gel Stain, 10,000× (Biotium, Fremont, California, USA). Negative controls were included in all reactions. Gels were visualized and photographed on a Bio-Rad transilluminator (Kodak Gel Logic 212 Imaging System). Five samples per population were sequenced at Macrogen, Korea. Sanger sequencing was performed with BigDye® Terminator chemistry on an ABI PRISM 3730 XL Analyzer (Applied Biosystems, California, USA).

Sequence editing and alignment

Consensus sequences were obtained using Staden Package version 1.6-r (Bonfield et al., 1995). BioEdit version 7.0.5 (Hall, 1999) was used for sequence analysis and alignment, using the CLUSTAL W Multiple Alignment method version 7.0.5 (Thompson et al., 1994). Polymorphic sites, nucleotide composition, number and diversity of haplotypes (COI fragments), and sequence variants/genotypes (ITS1 and ITS2) were calculated using DnaSP version 6.0 (Rozas et al., 2017).

Dataset

All sequences obtained in this study were deposited in GenBank (Table S3) and compared with ERM sequences to confirm species identity (Toda et al., 2000; Matsuda et al., 2014). Before inclusion in the final dataset, sequences retrieved from GenBank (Benson et al., 2013) and BOLD Systems (Ratnasingham et al., 2024) underwent taxonomic verification to confirm reliability by constructing a Neighbor-Joining (NJ) tree and calculating Kimura 2-parameter (K2P) distances (Austerlitz et al., 2009; Wiemers & Fiedler, 2007; Hebert et al., 2003).

The two selected COI fragments were extracted from the complete mitochondrial genome of ERM (NC_012571; EU682403) and included as reference sequences in phylogenetic analyses (Van Leeuwen et al., 2008). Similarly, one sequence from each COI fragment was extracted from the complete mitochondrial genome of *Panonychus citri* (McGregor) (HM189212) (Yuan et al., 2010) and included as an ingroup taxon. *Tetranychus urticae* (EU345430) (Van Leeuwen et al., 2008) served as the outgroup for both mitochondrial fragments.

Three ITS1 sequences were included in the analyses: *P. ulmi* (GQ141941), *P. citri* (HQ007221), and *T. urticae* (HM565875). For ITS2, the dataset was supplemented with 19 *P. ulmi* sequences (DQ656460–DQ656466, FJ997616, FJ969822–FJ969828, JF776969, KC416643, GQ141930, MW530359), two *P. citri* sequences (AM408034, DQ656444), and one *T. urticae* sequence (HM565874).

In addition to NCBI searches, a complementary search was conducted on the BOLD Systems database to ensure that all publicly available sequences were evaluated. Initially, 274 ERM sequences were retrieved; however, following metadata inspection and dataset curation, duplicates and non-standard regions were excluded. Because the remaining sequences lacked host information, they were omitted from phylogenetic and species delimitation analyses but were retained for haplotype collapsing to identify matches with our specimens. Detailed information on public database sequences included in this study is provided in Table S8. The term “lineage” was used to designate the different ERM populations evaluated across the phylogenetic and molecular analyses.

Phylogenetic analyses

Phylogenetic analyses were performed using Molecular Evolutionary Genetics Analysis version 7.0 (MEGA) (Kumar et al., 2016). Initially, the best nucleotide substitution model was tested and selected using AIC and the Bayesian information criterion (BIC) for each fragment in Smart Model Selection (SMS) in PhyML (http://www.atgc-montpellier.fr/sms/) (Lefort et al., 2017). The proportion of invariable sites (I), transition/transversion ratio (ti/tv), and gamma-distributed rates (G) were retained in all models. Best-fit models, AIC and BIC values, and nucleotide composition and frequencies are shown in Table S4.

Phylogenetic trees for the four fragments were estimated by maximum likelihood (ML) using MEGA 7.0. To determine confidence limits for branches generated in phylogenetic reconstruction, data were subjected to bootstrap testing (Felsenstein, 1985) with 10,000 replicates, and values were presented as percentages near tree branches. Bootstrap values below 50% were not shown. Shared haplotypes and genotypes were identified using DnaSP v.6 (Rozas et al., 2017).

For tree construction, one representative sequence from each sample was included in the alignment. When identical sequences (haplotypes and genotypes) were found, that is, sequences sharing a

common haplotype or genotype, one sequence from each group was included, and the number of identical sequences in the dataset was indicated in parentheses in the phylogenies.

Combined analysis

Mitochondrial and nuclear sequences (four fragments) were concatenated into a single matrix (59 taxa, 2,995 base pairs) based on the mite populations included in the output files of the ML analyses. The matrix was generated using Mesquite v.3.31 software (Maddison & Maddison, 2021) and the combined analysis was performed in MrBayes v.3.2.6 (Ronquist et al., 2012). The Bayesian concatenated analysis was performed under a GTR + I + G substitution model for all partitions, assuming a discrete gamma distribution with four rate categories. Model parameters and substitution rates were unlinked across partitions (ratepr=variable). Four Markov chains were run for 10,000,000 generations and sampled every 1,000 generations. The first 25% of the sampled trees (2,500,000 generations; 2,500 trees) were discarded as burn-in. Convergence was confirmed by an average standard deviation of split frequencies below 0.01.

Species delimitation

Lineage differentiation between apple and grapevine populations and genetic divergence among populations were assessed for all four fragments using a combined approach integrating barcode gap analysis, phylogenetic reconstruction, and genetic distance estimation. Lineage hypotheses were explored using ASAP (Assemble Species by Automatic Partitioning) (Puillandre et al., 2021) via the iTaxoTools web server (https://itaxotools.org/). Simple distance *(p*-distance) was selected as the substitution model for distance computation.

Inter and intraspecific genetic distances

Distances were calculated in MEGA 7 using the *p*-distance model, with standard error estimates obtained through bootstrap analysis (1,000 replicates), and the remaining parameters set to default. The uncorrected *p*-distance is widely used in DNA barcoding studies (Collins et al., 2012) because it requires minimal assumptions about nucleotide substitution patterns (Strimmer et al., 2009; Srivathsan & Meier, 2012), providing a straightforward measure of raw sequence divergence for species delimitation based on genetic gaps.

Clades revealed by Bayesian combined analysis and ML phylogenies, together with population groupings identified by ASAP, guided distance matrix calculations for the four fragments. For each proposed group, mean intraspecific distances (within-lineage diversity) and mean interspecific distances (between-lineage divergence) were calculated.

Maximum and minimum values within species (intraspecific variation) and between species (interspecific variation) were also determined. Minimum interspecific variation was considered the "Nearest Neighbor" distance following Kekkonen et al. (2015). These values were used to assess genetic separation between apple and grapevine populations and evaluate congruence between morphometric and molecular evidence.

Reproductive compatibility

ERM stock colonies

Two ERM stock colonies, one from apple (*M. domestica*) and one from grapevine (*V. vinifera*) (Table 1), were established from specimens collected between November 2014 and March 2015. All collected specimens were maintained in arenas prepared with apple leaves (grown without pesticide application in greenhouses) at Univates. Colonies were kept isolated from each other under controlled conditions in a climate chamber (25 ± 3°C, 14L:10D, and 75 ± 5% RH). The rearing methodology was adapted from Herbert (1981) and Johann (2014).

Crosses between mites from apple trees and grapevines

To facilitate the experiment, apple leaves were used as a common substrate for both colonies and for all crosses, owing to their greater longevity relative to grapevine leaves. All mites used in crosses were reared on apple leaves for a minimum of two generations to minimize stress associated with host switching.

To test the hypothesis of differential reproductive performance, a factorial design with six cross combinations (C1 to C6) was implemented. These included pairings of females and males from the same host plant, from different host plants, and isolated females (parthenogenesis) (Table 2).

Tests were conducted under the same conditions as the stock colonies. Each pair consisted of one deutonymphal female and one adult male from the respective stock colonies, totaling 15 pairs for each cross (C1, C2, C5, C6), plus 15 isolated females (C3, C4). Observations were conducted over eight days. Oviposition and mortality of each female were recorded daily. The experiment included two replicates with initially 30 pairs per cross, totaling 240 potential observations of reproductive performance per cross.

Table 2 Crosses and comparisons for the reproductive compatibility tests of two *Panonychus ulmi* (ERM) populations, from apple and grapevine.

Crosses		**Comparisons between crosses**
C1	♀ apple × ♂ apple	C1 and C2
C2	♀ grape × ♂ grape	C3 and C4
C3	♀ apple (isolated)	C5 and C6
C4	♀ grape (isolated)	C1, C3, and C6
C5	♀ grape × ♂ apple	C2, C4, and C5
C6	♀ apple × ♂ grape	

♀: females; ♂: males

Data analysis

Generalized Linear Mixed Models (GLMM) were applied for statistical analysis of reproductive compatibility. The purpose was to examine the influence of cross type (independent variable) on egg number (dependent variable). GLMMs were constructed using a Poisson distribution with a logarithmic link function (link = 'log') to model the relationship between response and independent variables. Model structure incorporated optimization via the 'bobyqa' method, designed to find optimal parameter values in nonlinear optimization problems.

GLMMs were constructed using the glmer function from the *lme4* package (Bates et al., 2015). Statistical significance and other GLMM parameters were subsequently extracted using the 'Anova' function from the *car* package (Fox & Weisberg, 2019). Results are displayed using the *ggplot2* package (Wickham, 2016) and related packages. These statistical analyses and graphs were performed in R.

Results

Morphological analyses

PCA identified two components: the first, designated "dorsal setae" (v2, sc1, sc2, c1, c2, c3, d1, d2, e1, e2, f1, f2, h1), explained 32.4% of the variance (eigenvalue = 6.48), and the second, designated "genital shield setae" (*ag, g1, g2, ps1, ps2, h2, h3*), explained 17.4% of the variance (eigenvalue = 3.48) (Figure 2).

The host had a global effect on both PCA components (dorsal setae: $\chi^2 = 41$; df = 8; $p < 0.001$; genital shield setae: $\chi^2 = 172$; df = 8; $p < 0.001$). For dorsal setae, populations from *V. vinifera* – Trebbiano (Pu4 and Pu11) had shorter setae than those from *V. vinifera* – Merlot (Pu2, Pu5, Pu6, and Pu7) (Estimate: −1.15; 95% CI: −1.68 to −0.62; $p < 0.001$).

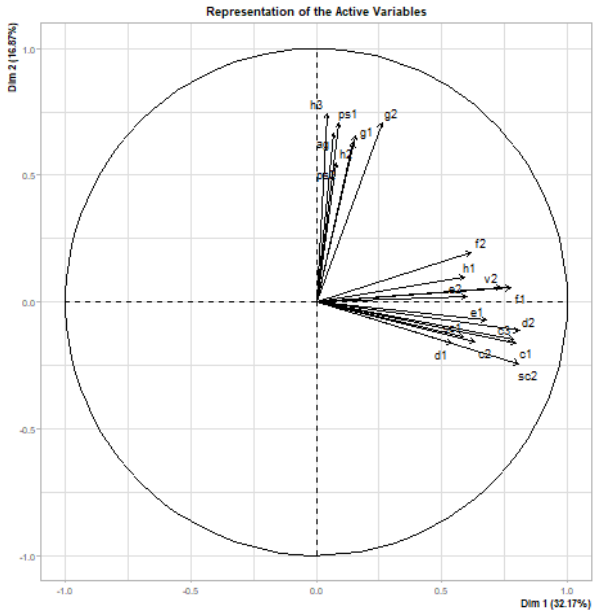


Fig. 2 Biplot generated by Principal Component Analysis (PCA) showing dorsal setae (v2, sc1, sc2, c1, c2, c3, d1, d2, e1, e2, f1, f2, h1) and genital shield setae (*ag, g1, g2, ps1, ps2, h2, h3*)

For *M. domestica* (Pu19.1), dorsal setae were shorter than in populations from *V. vinifera* – Cabernet Sauvignon (Pu8) (Estimate: 1.45; 95% CI: 0.63 to 2.26; $p < 0.001$). Among all *M. domestica* populations sampled, no statistical difference was observed in dorsal setae measurements.

Regarding genital shield setae, populations from *V. vinifera* – Merlot (Pu2, Pu5, Pu6, Pu7) had shorter setae than those from *M. domestica* – Fuji (Pu10) (Estimate: 1.07; 95% CI: 0.67 to 1.45; $p < 0.001$), *M. domestica* – Gala (Pu12, Pu13, Pu22.2) (Estimate: 1.12; 95% CI: 0.8 to 1.44; $p < 0.001$), and *M. domestica* (Pu19.1) (Estimate: 1.98; 95% CI: 1.47 to 2.49; $p < 0.001$).

When *M. domestica* (Pu19.1) was used as reference, the discrepancy in genital shield setae measurements across other hosts was larger than for dorsal setae. Population Pu19.1 had significantly higher values ($p < 0.05$) than all other hosts, except *V. labrusca* (Pu25.1) and *M. domestica* – Fuji (Pu10).

Notably, Pu19.1 had higher values than *M. domestica* – Gala (Pu12, Pu13, Pu22.2) (Estimate: −0.85; 95% CI: −1.36 to −0.35; $p = 0.001$), *V. vinifera* – Table grape (Pu3) (Estimate: −1.34; 95% CI: −2.00 to −0.68; $p < 0.001$), *V. vinifera* – Trebbiano (Pu4, Pu11) (Estimate: −2.17; 95% CI: −2.73 to −1.61; $p < 0.001$), *V. vinifera* – Cabernet Sauvignon (Pu8) (Estimate: −2.13; 95% CI: −2.75 to −1.50; $p < 0.001$), and *V. vinifera* – Pinot Noir (Pu9) (Estimate: −2.74; 95% CI: −3.36 to −2.12; $p < 0.001$).

In the host type model run without cultivar differentiation, no significant difference was found for dorsal setae ($\chi^2 = 1.32$; df = 1; $p = 0.25$). However, genital shield setae showed morphometric differences ($\chi^2 = 80.1$; df = 1; $p < 0.001$), with apple populations having larger measurements than grapevine populations (Estimate = 1.19; 95% CI: 0.93 to 1.45; $p < 0.001$).

Molecular characterization

Datasets, haplotypes/genotypes, and sequence diversity indexes

Species assignments, DNA sequence variation (haplotypes, genotypes), sequence frequencies in the dataset (frequency), and respective GenBank accessions are presented in Tables S3 and S8.

COI barcoding

The dataset comprised 95 sequences (686 bp) with an average nucleotide composition of 46.5% T, 31.9% A, 11.3% C, and 10.3% G (78.4% A+T, 21.6% C+G). The final alignment included 92 newly generated ERM sequences and three GenBank references.

Eight haplotypes (H01–H08) were initially identified in the *Panonychus* sequences. H01 was the most frequent, with 22 sequences (23.65% of the total): 21 from apple trees (BRA, JPN, MAR, and ZAF) and one from grapevine (BRA), Pu25-1d (*V. labrusca*). H03 grouped 18 sequences (19.36%) from mixed populations collected on both apple trees and grapevines (BRA), plus populations from apple trees (MAR, ZAF). H02 and H06 grouped 21 (22.58%) and seven (7.53%) ERM sequences, respectively, exclusively from grapevines in Brazil. H04 grouped 14 sequences (15.05%) from populations on pear (JPN) and apple (USA and ZAF). Populations from Chile (apple and almond) formed H05 and H08 (Table S3).

COI Navajas

The dataset comprised 98 sequences (892 bp) with an average nucleotide composition of 45.3% T, 31.2% A, 11.5% C, and 12.0% G (76.5% A+T, 23.5% C+G). The final alignment consisted of 96 newly generated ERM sequences and two GenBank references.

The sequences were also divided into eight haplotypes. H01 and H03 grouped sequences from populations collected on both apple trees and grapevines across different countries. H01 comprised 22 sequences (22.915%) from apple (BRA, JPN, MAR, and ZAF) and grapevine (BRA) populations. H03 grouped 19 sequences (19.79%) from apple trees (BRA and ZAF) and grapevines (BRA). In contrast, H02 and H06 were formed exclusively by grapevine populations from Brazil, containing 21 and seven sequences, representing 21.87% and 7,29% of the total, respectively. H04, with 15 sequences (15.62%),

consisted of samples from apple trees (USA and ZAF) and pear (JPN). Populations from Chile collected on apple and almond (10 sequences; 10.410%) formed H05, completing the *COI* haplotype set (Table S3).

ITS1 region

The ITS1 dataset comprised 76 sequences (~583 bp) with an average nucleotide composition of 31.05% T, 31.56% A, 18.52% C, and 18.87% G (62.61% A+T, 37.39% C+G). The final alignment consisted of 73 newly generated ERM sequences and three GenBank references.

The ITS1 dataset was divided into 26 genotypes (G01–G26). G01 grouped 38 sequences (51.35% of the total) from apple trees (BRA, CHL, MAR, JPN, USA, and ZAF), pear (JPN), and almond (CHL). G02 comprised samples collected in Brazil from apple trees and grapevines. G03 and G04 were formed by grapevine samples, with G03 consisting of sequences from *V. vinifera* grapevine (Pu21) and G04 from *V. labrusca* grapevine (Pu25). G05 through G26 were represented by individual sequences from grapevine populations in Brazil (Table S3), except for one sequence from China collected on apple trees (GQ141941).

ITS2 region

The dataset comprised 104 sequences (~760 bp) with an average nucleotide composition of 30.96% T, 29.07% A, 18.33% C, and 21.64% G (60.03% A+T, 39.97% C+G). The final alignment included 82 newly generated ERM sequences and 22 GenBank references.

Nine genotypes were identified (G01-G09). G01 grouped 57 sequences (56.44%) from mixed populations: 48 from apple trees (BRA, MAR, JPN, USA, and ZAF), six from almond (CHL), three from pear (JPN). G02 comprised populations collected on different hosts: grapevine (25 sequences – BRA), apple (4 – BRA and CHL), and pear (5 – JPN), totaling 34 sequences (33.66%). See Table S3 for complete haplotype/genotype distribution and Table 1 for the list of hosts and collection localities.

Genetic diversity indices

Intraspecific genetic diversity indices were estimated for each fragment. Nucleotide diversity (*Pi*) varied from 0.28% (ITS2) to 1.38% (COI barcoding). The mitochondrial fragments showed a range of 1.13% to 1.38%, while the nuclear fragments ranged from 0.28% to 0.97% (Table S5). *Pi* for COI (≈0.01378) was approximately five times higher than that observed for ITS2 (≈0.00275), consistent with the relative mutation rate observed for mtDNA in invertebrates (Allio et al., 2017). The average number of nucleotide differences *(K*) is directly proportional to Pi. Mean *K* values for mitochondrial fragments were also higher than those for the nuclear fragment ITS2.

Combined phylogenetic trees based on all the tested fragments

The Bayesian inference (BI) analysis of the concatenated dataset (Bayesian combined analysis) showed that *Panonychus* samples formed a monophyletic group (Bayesian Posterior Probability = 1.0). Samples initially identified as ERM were consistently separated from *P. citri* (BPP = 1.0) The different haplotypes/genotypes of ERM grouped into two well-supported major clades (BPP = 1.0) (Figure 3).

The first clade (Group I, hereafter GI) comprised mixed populations from apple trees, grapevines, and pear, originating from various parts of the world (BRA, JPN, MAR, USA, ZAF) sampled in this study.

Within this clade (GI), populations collected from grapevines in RS, Brazil (BBP = 0.72) remained separate from other populations collected from apple trees across different regions of the world and from pear (JPN), forming an isolated subclade. The second clade (Group II, hereafter GII) comprised populations (Pu20 and Pu28) collected in Chile (BPP = 1) from almond and apple trees.

The topologies of the phylogenetic trees inferred independently by the NJ, ML, and BI methods for the four fragments were congruent and revealed a highly similar pattern. Accordingly, only the individual phylogenies constructed by the ML method are presented for each fragment (Figures S1 to S4).

Molecular data analysis and species delimitation

ASAP analysis of the COI barcoding fragment yielded two partitions within the barcode gap interval, with a threshold of d*T* = 0.027697 (Prob: 2.463768e-02) falling within the 0.005–0.05 range. Among the proposed partitions, the one with the highest W value (gap width = 7.20e−05) (rank = 3) (ASAP-score: 2.500000) was selected as the best candidate to represent biologically plausible lineage boundaries. These boundaries corresponded to the most evident gaps within the expected divergence range for lineages in this partition. Two groups were identified: 1) GI = ERM collected from apple trees (BRA, JPN, MAR, USA, ZAF), pear (JPN), and grapevines (BRA); 2) GII = specimens from apple and almond (CHL). These groups coincide with the clades recovered in the concatenated phylogeny, indicating concordance between the two methods employed.

Analysis of the *COI* Navajas dataset identified a single partition within the barcode gap, with ERM sequences grouping in exactly the same pattern observed for *COI* barcoding (GI and GII): d*T* = 0.026741, Prob = 1.822731e-02, W (rank) = 4.90e−05 (2), and ASAP-score = 2.500000.

The nuclear regions did not isolate the Chilean samples as observed with the two mitochondrial markers. ITS1 grouped all dataset sequences except four grapevine samples from Brazil (Trebbiano and Pinot Noir: Pu9_4, Pu9_6, Pu9_8, and Pu4_8), which formed a separate group. ASAP parameters for ITS1 were: d*T* = 0.011149, Prob = 4.11e−01, W (rank) = 3.35e−05 (3), and ASAP-score = 5.00000. ITS2 grouped all sequences in the dataset into a single cluster, except for KC416643. ASAP parameters for the ITS2 region, first partition: dT = 0.034205, Prob = 9.999900e−06, W (rank) = 1.00 (1), and ASAP-score = 1.

Species-level divergence and genetic distances in *Panonychus* populations

The overall mean divergence among ERM populations based on COI barcoding was 1.38% (SE = 0.0027), ranging from 0.0% to 5.89%. Pairwise distances within and between ERM groups, its congeneric *P. citri*, and the outgroup are presented in Table S6. Using COI Navajas, average divergence was 1.27% (SE = 0.0018; range: 0.00–5.24%). For the ITS1 and ITS2 regions, average divergence among ERM genotypes was 1.02% (SE = 0.003; range: 0.1–5.33%) and 0.16% (SE = 0.004; range: 0.00–1.06%), respectively. Divergence between the two main clades (GI and GII) in the concatenated tree was 2.2% (SE = 0.5%), and within-group divergence was 0.5% (GI) and 0.2% (GII).

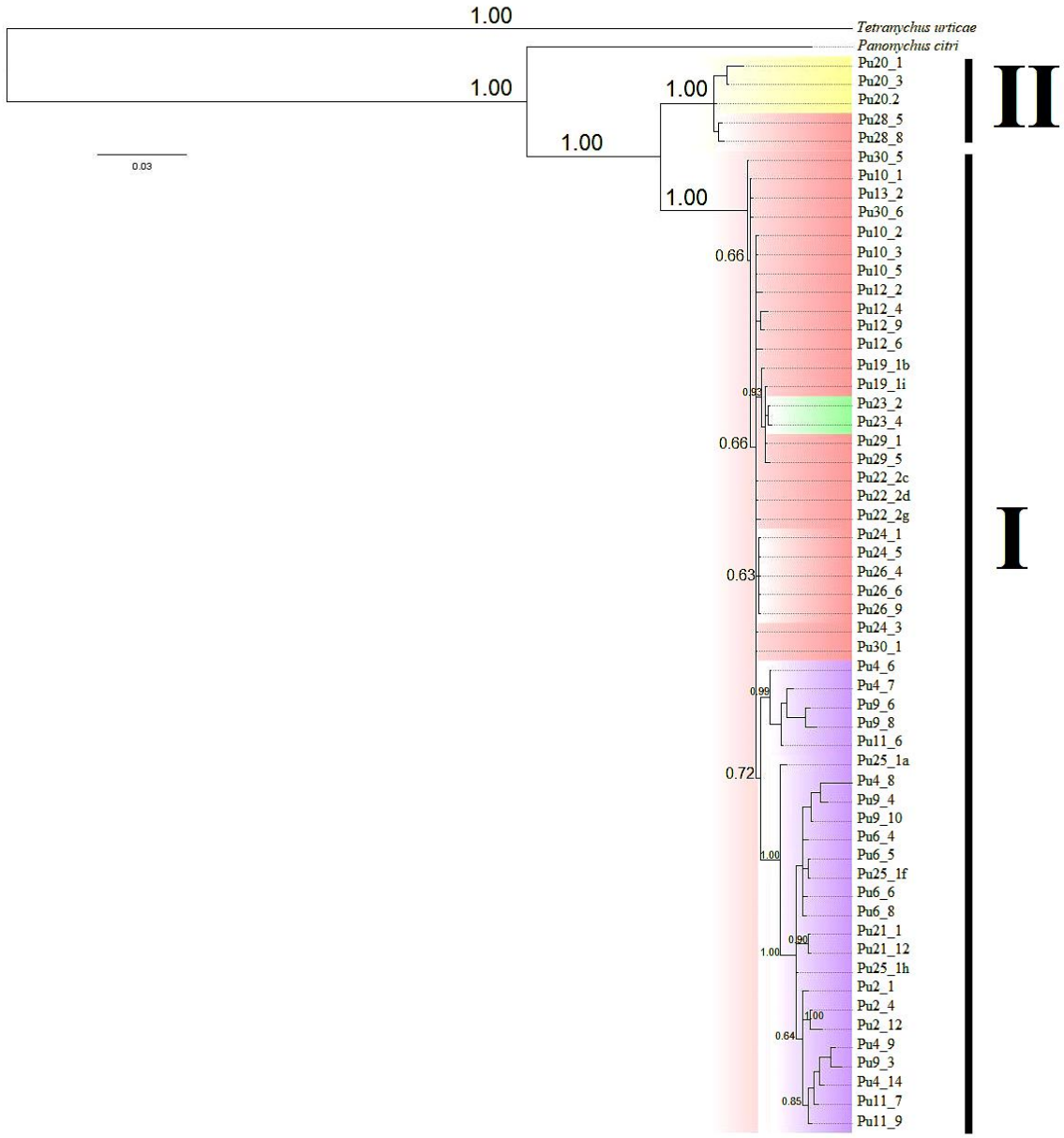


Fig. 3 Bayesian Inference (BI) phylogenetic tree of *Panonychus ulmi* (ERM) based on a combined analysis of four genomic regions: the mitochondrial cytochrome c oxidase subunit I (COI barcoding and COI Navajas) and ribosomal internal transcribed spacers 1 and 2 (ITS1 and ITS2). Bayesian posterior probabilities are shown above branches; values < 0.60 are not shown. *Panonychus ulmi* (ERM) populations are color-coded by host plant: red = apple; purple = grapevine; yellow = almond; green = pear. Groups I and II are indicated by I and II, respectively. *Panonychus citri* and *Tetranychus urticae* were used as the sister group and outgroup, respectively

Genetic distance matrices (pairwise comparisons) within and between *Panonychus* populations and the outgroup are presented in Tables S6 and S7 for all four fragments. Phylogenetic results (concatenated and individual trees) and ASAP partitioning of the mitochondrial fragments informed the groupings used to construct the distance matrix (Table S6): GI, comprising mixed ERM populations from apple, pear, and grapevine collected worldwide (Table 1), and GII, comprising apple and almond populations from Chile (CHL).

The mean distance between Groups I and II was 5.16% (min = 4.71%; max = 5.89%) for COI barcoding and 4.97% (4.74–5.24%) for COI Navajas. Intraspecific values within GI were 0.56% (0.00–1.33%) for COI barcoding and 0.41% (0.00–0.99%) for COI Navajas. Interspecific distances between ERM and *P. citri* were 7.85% and 9.08% for COI barcoding and COI Navajas, respectively (Table S6).

For the ITS1 and ITS2 markers, neither ASAP nor the individual phylogenetic reconstructions resolved the Chilean apple and almond samples as a genetically distinct lineage. Consequently, in the distance matrix (Table S7), grapevine, apple, pear, and almond populations were pooled into a single nuclear group (Group I–ITS, hereafter GI–ITS). Mean intraspecific genetic distances within this group were 0.64% (min = 0.00%; max = 2.46%) for ITS1 and 0.16% (0.00–1.06%) for ITS2.

Two exceptions were noted in the nuclear distance calculations. The first involved four ITS1 sequences (Pu9_4, Pu9_6, Pu9_8, and Pu4_8) that remained isolated under the ASAP partition. Mean pairwise distance among these genotypes was 2.45% (0.61–3.89%), slightly above the range typically defined for intraspecific boundaries; they were therefore treated as outliers.

The second exception concerns ITS2 accession KC416643, which was similarly isolated from GI–ITS populations by ASAP. The mean distance between KC416643 and GI–ITS was markedly higher, at 5.22% (5.01–5.54%; Table S7).

The subclade composed exclusively of grapevine ERM populations, recovered within GI in the combined phylogeny, was also recovered in the individual mitochondrial phylogenies, where grapevine populations showed partial separation (Figures S1–S2). Despite this internal structuring, mean intraspecific variation remained low: 0.56% for COI barcoding and 0.41% for COI Navajas.

Reproductive compatibility

In cross C1 (♀ apple × ♂ apple), 704 eggs were recorded (mean 28.16 eggs/female ± 13.59 SE), and in C2 (♀ grapevine × ♂ grapevine), 467 eggs (20.30 ± 11.02 SE). Mean daily oviposition was 4.32 ± 0.19 for C1 and 3.89 ± 0.22 for C2. Although mean egg production in C1 was approximately 11% higher than in C2, the statistical model detected no significant difference between crosses (GLMM Poisson: χ^2 = 2.22, df = 1, p = 0.135; Table S9, Figure 4A) under the tested conditions.

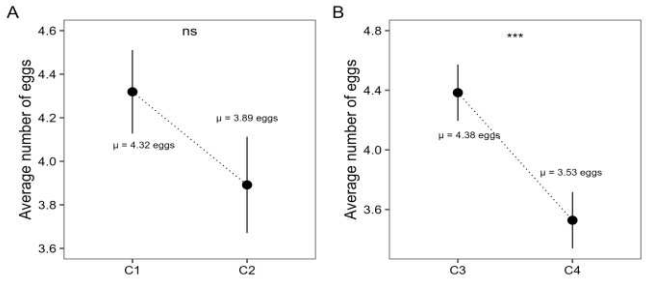


Fig. 4 Mean oviposition (μ) for the crosses and combinations analyzed. (A) Combinations C1 (♀ apple × ♂ apple) and C2 (♀ grapevine × ♂ grapevine); (B) Combinations C3 (♀ apple, isolated) and C4 (♀ grapevine, isolated). Note: Points represent mean, vertical bars indicate standard error, and the vertical line connects both experiments to facilitate data interpretation. The abbreviation *ns* indicates a non-significant difference, while asterisks denote statistically significant differences ($p < 0.001$)

In the experiment evaluating reproductive performance of isolated females from their respective host plants, C3 (♀ apple, isolated) yielded 754 eggs (30.16 ± 13.47 SE) and C4 (♀ grapevine, isolated) 441

eggs (19.17 ± 12.85 SE). Mean egg production by apple-derived females was approximately 24% higher than that of grapevine-derived females. This pattern was reflected in the daily oviposition rate, which was significantly higher in C3 (4.38 ± 0.18) than in C4 (3.53 ± 0.18) (GLMM Poisson: $\chi^2 = 11.1$, df = 1, $p < 0.001$; Table S10, Figure 4B), indicating that the female's host plant of origin influenced fecundity.

Among pairs from different host plants, C5 (♀ grapevine × ♂ apple) yielded 329 eggs (14.95 ± 10.07 SE) and C6 (♀ apple × ♂ grapevine) 728 eggs (29.12 ± 14.61 SE). Mean egg production differed significantly between crosses (GLMM Poisson: $\chi^2 = 10.6$, df = 1, $p = 0.001$; Table S11, Figure 5). Daily oviposition by apple-derived females crossed with grapevine males (C6) was 37% higher (4.52 ± 0.22) than that of grapevine-derived females crossed with apple males (C5) (3.29 ± 0.20), further indicating an effect of the female's host plant of origin.

Comparing crosses involving apple-derived females (C1, C3, and C6), total egg production was 2,186 (C1 = 704, 32.2%; C3 = 754, 34.5%; C6 = 728, 33.3%), with mean daily oviposition of 4.32 ± 0.19 (C1), 4.38 ± 0.18 (C3), and 4.52 ± 0.22 (C6) (Figure 6A).

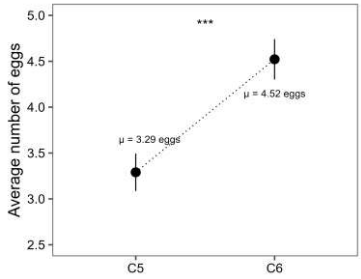


Fig. 5 Mean oviposition (μ) for combinations C5 (♀ grapevine × ♂ apple) and C6 (♀ apple × ♂ grapevine). Note: Points represent mean, vertical bars indicate standard error, and the vertical line connects both experiments to facilitate interpretation. Asterisks indicate statistically significant differences ($p = 0.001$)

No significant difference in mean egg production was detected among these crosses (GLMM Poisson: $\chi^2 = 0.86$, df = 2, $p = 0.648$; Table S12, Figure 6A). This result suggests that in combinations involving apple-derived females, male origin had no positive or negative effect on female reproductive performance. Oviposition by apple-derived females was consistent across all crosses tested.

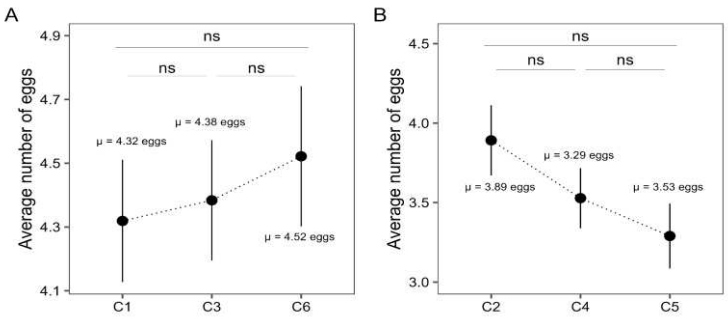


Fig. 6 Mean oviposition (μ) for the crosses and combinations analyzed. (a) C1 (♀ apple × ♂ apple), C3 (♀ apple, parthenogenesis), and C6 (♀ apple × ♂ grapevine). (b) C2 (♀ grapevine × ♂ grapevine), C4 (♀ grapevine, parthenogenesis), and C5 (♀ grapevine × ♂ apple). Note: Points represent mean, vertical bars indicate standard error, and the vertical line connects the experiments to facilitate interpretation. The abbreviation *ns* indicates no statistically significant difference between the pairs compared

Comparing crosses involving grapevine-derived females (C2, C4, and C5), total egg production was 1,237 (C2 = 467, 37.8%; C4 = 441, 35.7%; C5 = 329, 26.6%), with mean daily oviposition of 3.89 ±

0.22 (C2), 3.53 ± 0.18 (C4), and 3.29 ± 0.20 (C5) (Figure 6B). As in the previous comparison, no significant difference was detected among these crosses (GLMM Poisson: $\chi^2 = 4.23$, df = 2, $p = 0.127$; Table S13, Figure 6B). Oviposition by grapevine-derived females was consistent across all crosses tested. Table 3 presents egg viability and sex ratio for all crosses.

Table 3 Results of reproductive compatibility analyses of two *Panonychus ulmi* (ERM) populations from apple trees and grapevine.

Crosses	n	Total eggs	Eggs/female	Eggs/female/day	Egg viability	Sex ratio
C1 ♀ apple × ♂ apple	25	704	28.16±13.59	4.32±0.19	0.90±0.14	0.75±0.43
C2 ♀ grapevine × ♂ grapevine	23	467	20.30±11.02	3.89±0.22	0.87±0.27	0.55±0.37
C3 ♀ apple isolated	25	754	30.16±13.47	4.38±0.18	0.91±0.09	-
C4 ♀ grapevine isolated	23	441	19.17±12.85	3.53±0.18	0.74±0.34	-
C5 ♀ grapevine × ♂ apple	22	329	14.95±10.07	3.29±0.20	0.98±0.05	0.90±0.15
C6 ♀ apple × ♂ grapevine	25	728	29.12±14.61	4.52±0.22	0.99±0.02	0.68±0.14

♀: females; ♂: males; values presented as mean ± standard error; *n* = number of pairs or isolated females used in the calculation; sex ratio expressed as proportion of females

Isolated females produced only males. All crosses between males and females, regardless of host plant of origin, were viable and produced females. This result suggests that, as with apple-derived females, male origin had no significant effect on the reproductive performance of grapevine-derived females.

Discussion

Panonychus ulmi (ERM) was first recorded in Brazil by Flechtmann (1967) on apples imported from Argentina. The species was subsequently reported in orchards in Santa Catarina (Bleicher, 1974), and, although it spread to other states in southern Brazil, it remained restricted to apple for decades. During that period, ERM was established in the country but, if present on grapevines, went undetected in vineyard surveys. This scenario differed from the pattern observed in countries such as France, Austria, Portugal, Italy, and Greece, where the species has historically been among the primary pests of viticulture (Rambier, 1958; Carmona & Dias, 1980; Papaioannou-Souliotis et al., 1994).

The risk of introduction of European biotypes adapted to grapevine, possibly via transport of winter eggs on infested canes, had already been flagged (Moraes & Flechtmann, 2008; Mendonça et al., 2011). Host transfer was confirmed in Brazil with the first records on grapevines in RS during the 2005–2006 season (Ferla & Botton, 2008; Johann et al., 2009), and subsequently in Pirapora, Minas Gerais (Mendonça et al., 2011).

The expansion of ERM onto grapevines in Brazil raised the possibility of host adaptation or the introduction of new genetic variants. However, the morphometric, molecular, and reproductive compatibility data presented here do not support either hypothesis. Although adaptation to novel hosts can promote genetic differentiation and host-associated divergence (Weeks et al., 2000; Dermauw et al., 2013), the populations examined share a common biological identity, with no detectable genetic or taxonomic divergence associated with specific hosts.

Morphological analyses

The extent of morphological variation detected depended on the characters examined. No consistent differences in dorsal setae were observed between host types, suggesting a greater influence of local factors on these structures. In contrast, genital shield setae showed significant differentiation, with apple-tree populations exhibiting a higher probability of greater values relative to grapevine populations. We therefore conclude that morphometric differentiation exists between ERM populations from different hosts, though restricted to specific characters. The host plant and local environment appear to act as modulators of morphology and reproductive potential (Johann et al., 2019; Meghana et al., 2023; Kasap & Atlihan, 2021), without producing a uniform pattern of variation sufficient to support taxonomic separation (Grbic et al., 2011).

The influence of habitat on morphology is particularly relevant in mites associated with different hosts or exploiting multiple food substrates (Klimov et al., 2004). Studies have shown that intraspecific variability in metric variables can be influenced by factors such as environmental conditions, geographic region, and laboratory rearing conditions (Ebermann et al., 2013).

The high similarity among the analyzed populations suggests low divergence between them. Given that this represents a recent introduction, the time since population establishment may not have been sufficient for greater morphometric differentiation to occur (Navia et al., 2009).

Molecular characterization

The best partition identified by ASAP clustered COI sequences of ERM populations into two distinct groups (GI and GII), which was also supported by the combined phylogeny (Figure 3). Genetic distances between these groups for the COI barcoding (5.16%) and COI Navajas (4.97%) fragments exceeded the conservative interspecific divergence thresholds (2.7% to 3.0%) established for species delimitation (Hebert et al., 2003; Barrett & Hebert, 2005).

The mean intraspecific variation values for both mitochondrial fragments (GI: 0.56% and 0.41%; GII: 0.03% and 0.00%) were lower than the 1.1% threshold reported for ERM (İnak et al., 2022), confirming the internal consistency of each cluster. Furthermore, analysis of sequences in BOLD Systems revealed 14 specimens from Canada and one from Russia with haplotypes matching those of the Chilean populations, all clustering as H05 (Table S8). The intraspecific variability of GII (Chile + Canada + Russia, COI barcoding) was 0.13%, indicating strong genetic cohesion and confirming the stability of this lineage across different geographical regions.

The literature reports minimum interspecific divergences within the genus *Panonychus*, such as 8.8% (*P. ulmi* vs. *P. caglei*; Khaing et al., 2015) and 8.9% (*P. ulmi* vs. *P. citri*; Guo-Qing et al., 2010). The values found in the present study (7.85% and 9.08%; Table S6) approach these ranges. The magnitude of the separation detected for GII becomes even more evident when compared to exceptional cases in Tetranychidae, such as the low distance of 1.9% separating morphologically distinct species like *T. urticae* and *Tetranychus turkestani* (Ugarov & Nikolskii, 1937) (İnak et al., 2022). The mitochondrial divergence detected here therefore points to the existence of a distinct ERM lineage occurring on apple and almond trees in Chile, and collected via traps in Canada and Russia, warranting morphological studies to elucidate the taxonomic status of this population.

Conversely, the partition analysis of nuclear markers via ASAP delimited a single group (GI–ITS), consistent with findings from the individual phylogenies (see Figures S3 and S4). The low observed intraspecific divergence (ITS1, 0.64%; ITS2, 0.16%) was consistent with the 0.2% to 0.6% range reported by Ben-David et al. (2007) for ERM. Regarding interspecific divergence, distances between ERM and *P. citri* (ITS1, 9.58%; ITS2, 9.75%) fell within the range described for Tetranychidae. These values were intermediate compared to those reported by Li et al. (2010), who observed 8.15% (ITS1) and 6.74% (ITS2), and Khaing et al. (2015), who recorded 13.3% for ITS2. This variation in the literature (6.74%–13.3%) suggests the influence of geographical origin or the intrinsic variability of each lineage.

Thus, the mitochondrial sequences clustered in GI, together with the nuclear markers, evidenced a lineage adapted to both apple and grapevine as it spread gradually across Brazil, as well as apple and pear trees in South Africa, the United States, Japan, and Morocco. While the nuclear data showed homogeneity among populations, the mitochondrial markers revealed that populations from Chile, Canada, and Russia exhibit genetic differentiation, forming a lineage with distinct molecular characteristics. This divergence between markers highlights the importance of investigating whether such mitochondrial isolation reflects cryptic speciation or a particular evolutionary event within this lineage. This scenario will be further explored in the subsequent sections.

Mitochondrial vs. nuclear loci: contrasting evolutionary rates and nucleotide composition

The nucleotide composition of the mitochondrial fragments is consistent with the patterns described for Tetranychidae, with a predominance of A+T (Fournier *et al.*, 1994; Navajas *et al.*, 1996; Ros & Breeuwer, 2007; Matsuda *et al.*, 2012). Similarly, the ITS1 and ITS2 regions were A+T-rich. Although direct comparisons of A+T content between these two nuclear markers are lacking in the literature, the results obtained in this study are in line with the general patterns described for eukaryotes (Wang *et al.*, 2015).

The mitochondrial fragments showed greater *Pi* values than the nuclear loci, notably ITS2. This differential mutation rate between genomes is expected, as mtDNA tends to evolve faster per generation than nuclear DNA (Allio et al., 2017). The inherently higher mutation rate of *COI* markers (Gollner et al., 2016) allows for more rapid accumulation of polymorphisms, making this marker sensitive to genetic differences at recent geographic and temporal scales, as previously observed in other invertebrates (Matthee et al., 2020; Gollner et al., 2016). Consequently, the allelic diversity represented by the *Pi* and K metrics observed for *COI* suggested that this marker was more robust for phylogenetic and population structure analyses, whereas ITS2 was less variable.

Species delimitation: single lineage expansion vs. Chilean divergence

The combined phylogeny revealed a subclade composed exclusively of grapevine populations within GI (Figure 3). The mean genetic distance (p-distance) between this subclade and the clade formed by mixed populations (apple and pear trees) within GI was 1.10% (0.89–1.34%) for the *COI* barcoding and 0.80% (0.63–1.01%) for *COI* Navajas fragments. These values are too low to support species separation (Avise, 2000; Guo-Qing Li et al., 2010; İnak et al., 2022) and are comparable to intraspecific distance values, suggesting the absence of host-associated biological structuring.

The mitochondrial markers also indicated that the Chilean populations (Pu20 and Pu28) represent a distinct ERM lineage. High divergences typically occur in geographically isolated populations or in cases of taxonomic uncertainty, where distinct lineages share the same specific epithet despite representing different biological entities (Avise & Walker, 1999). Neither nuclear marker corroborates this distinction, indicating different evolutionary histories between the mitochondrial and nuclear genomes (Toda et al., 2000).

This divergence characterizes the phenomenon of mitonuclear discordance, frequently attributed to introgression, incomplete lineage sorting, or asymmetric demographic histories between genomes (Després, 2019). Due to maternal inheritance and smaller effective population size, *COI* can reveal divergent lineages and fine-scale population structure, while nuclear markers subject to concerted evolution, such as ITS, tend to remain homogeneous (Navajas et al., 1998; Osakabe et al., 2005; Ros & Breeuwer, 2007; Ben-David et al., 2007).

In Hymenoptera, the *COI* gene has revealed well-structured lineages lacking equivalent differentiation at nuclear loci, a disparity driven by the smaller effective population size and higher mtDNA substitution rate (Rohde et al., 2025). This pattern of evolutionary asynchrony supports the results obtained here for Chilean *Panonychus* populations. An analogous phenomenon has been reported for mites of the genus *Brevipalpus*, where *COI* could detect host-associated lineages or cryptic species, suggesting that such inconsistency reflects recent speciation events, gene flow via introgression, or contrasting evolutionary rates between organellar and nuclear genomes (Mendonça et al., 2025). This phenomenon is consistent with a scenario of incipient divergence between the Chilean *Panonychus* lineage and ERM, initially detected in the mitochondrial genome but not yet leaving a clear signature in the nuclear markers. Confirmation of the taxonomic status of this taxon requires additional sampling in Chile, preferably including male specimens for complementary integrated morphological analysis.

ITS1 uncertainty: insights into genomic variation

The occurrence of atypical ITS1 variants in intraspecific datasets may be associated with genomic plasticity (Nyaku et al., 2024; Tang et al., 1996) or with intra-individual variation resulting from incomplete homogenization of rDNA copies (concerted evolution) (von der Schulenburg et al., 2001; Ritchie et al., 2004; Nyaku et al., 2024).

Alternatively, PCR artifacts such as template switching in repetitive regions may generate artificial length variants or sequences that do not reflect the predominant genomic composition (Fenton et al., 1998). In this context, Hurtado et al. (2008) recommended caution in the use of ITS1 for identification in Tetranychidae, given its excessive variability, which may indicate paralogous rDNA copies rather than genuine population diversity. A similar finding was reported for Phytoseiidae, where high variability in this region hampered alignment and limited its taxonomic utility (Navajas et al., 1996).

Accordingly, the atypical samples from grapevines in Bento Gonçalves (BRA), although morphologically indistinguishable, were treated separately to avoid distortions in divergence boundaries that could compromise species delimitation and barcoding analyses (Tang et al., 1996; Hurtado et al., 2008; Nyaku et al., 2024). In summary, the literature provides a solid basis for the occurrence of atypical ITS1

sequences in intraspecific datasets, reinforcing the need for caution in their interpretation and the adoption of integrative approaches for accurate molecular identification.

Overcoming taxonomic ambiguities in ITS2 sequences

The ITS2 region has been widely used in phylogenetic and species delimitation studies owing to its low intraspecific variability and the presence of fixed differences between taxa, particularly in the genera *Tetranychus* and *Panonychus* (Hurtado et al., 2008; Li et al., 2010). The homogeneity of ITS2 and its effectiveness in discriminating closely related species justify its application as a robust marker for molecular diagnostics, barcoding, and large-sample screening in Tetranychidae (Hurtado et al., 2008).

Although no universal threshold exists for species separation, the literature on ITS2 in Tetranychidae converges on an operational cutoff of approximately 2%, which Ben-David et al. (2007) found effective in segregating 14 of 16 species in the family. Values ≤ 1–1.2% fall within intraspecific variation for Tetranychidae (İnak et al., 2022; Mushtaq et al., 2023; Ben-David et al., 2007), while distances ≥ 3–4% are strongly indicative of distinct species (Mushtaq et al., 2023; Ben-David et al., 2007). The intermediate range of 1.5–3% remains ambiguous and should be interpreted in conjunction with phylogenetic, morphological, and additional marker data where available (Mushtaq et al., 2023; Ben-David et al., 2007; Matsuda et al., 2013).

In *Oligonychus*, for example, intraspecific ITS2 divergences range from 0% to 1.2%, contrasting with considerably higher interspecific values (3.7%–51.1%) (Mushtaq et al., 2023). In this context, the divergence of 5.22% found in the present study between sequence KC416643 and GI–ITS surpasses the intraspecific thresholds discussed above (Ben-David et al., 2007; İnak et al., 2022; Mushtaq et al., 2023) and falls within the range of interspecific divergence reported for Tetranychidae. One plausible explanation is that sequence KC416643 might stem from a misidentified specimen in GenBank.

Public DNA databases are known to harbor misidentified mite sequences, with cases documented in both Tetranychidae (Mendonça et al., 2011) and Phytoseiidae (Tixier et al., 2011). These inconsistencies highlight the critical role of rigorous taxonomic vouchering in underpinning reliable phylogenetic and molecular diagnostic studies. Sequences showing atypical genetic divergence relative to the reported taxon should therefore be treated with caution and interpreted within an integrative framework.

Reproductive compatibility

No comparative crossing studies between ERM populations from apple and grapevine have been published to date. This study expands knowledge of this species and its interaction with different hosts, showing that the female's host of origin was the primary determinant of reproductive performance: females reared on apple trees were more fecund than those from grapevines. Male presence and male origin, by contrast, had no significant effect on oviposition, suggesting that reproductive output is driven mainly by maternal factors tied to the host of origin.

These findings are consistent with earlier studies reporting feeding preference and superior performance of ERM on apple trees (Johann et al., 2019; Silva et al., 2021; Kasap, 2021) and support the hypothesis that host plant characteristics, particularly nutritional quality and leaf physical properties,

directly shape mite oviposition and development. While *V. vinifera* cultivars can sustain ERM populations, apple trees are the more favorable host, yielding higher reproductive potential.

Male origin had no effect on fecundity regardless of the cross combination, indicating no reproductive incompatibility between the populations tested. All crosses produced females, supporting reproductive compatibility, unlike some tetranychids in which crossing incompatibilities can result in reduced offspring viability or strongly male-biased progeny (Gotoh & Takayama, 1992; Gotoh et al., 1995). ERM populations from apple and grapevine therefore appear to lack reproductive isolation, notwithstanding potential host-driven adaptive differences.

Exclusive male production by unmated females confirms the arrhenotokous reproductive system of the species (Helle & Bolland, 1967), consistent across both populations. The fecundity differences observed are thus attributable to host-related adaptation rather than to any change in the reproductive system itself.

The feasibility of crosses between populations from different hosts, together with the absence of reproductive barriers, points to potential gene flow between apple-tree and grapevine populations. Where these crops coexist (as in southern Brazil), such connectivity may sustain population exchange between hosts across growing seasons, reinforcing the mite's persistence in the landscape. These dynamics, including crop connectivity and the adaptive plasticity of the species, should be factored into management strategies for regions where both crops are cultivated.

Conclusion

ERM populations tested in this study belong to the same species, as evidenced by morphological similarity, reproductive compatibility, and low intraspecific genetic distance. Although apple-tree populations showed higher oviposition efficiency and phylogenetic analysis suggested a grouping of grapevine samples, these findings were insufficient to support the recognition of separate species. The complexity of the interactions observed suggests that further research may be needed to elucidate the underlying dynamics of these reproductive outcomes in ERM.

Taken together, the integration of multiple diagnostic tools does not support the existence of ERM lineages strictly associated with these hosts in the analyzed samples. The low divergence observed (GI and GI–ITS), even when populations from diverse geographical origins were included, corroborates the patterns documented in the literature for a single species (Guo-Qing Li et al., 2010; İnak et al., 2022).

The observed genetic homogeneity is relevant to the Brazilian phytosanitary context. Since the first record of ERM in Brazilian orchards (Bleicher, 1974), only a single lineage has become established and has persisted. Initially associated with apple trees, this lineage (GI and GI–ITS) subsequently spread to grapevines in RS (2008) and Minas Gerais (Mendonça et al., 2011), with no evidence of sympatric speciation or host-based structuring.

Predictive modeling based on bioclimatic inferences indicates that the South region of Brazil offers higher environmental suitability for the species, while occurrence in the Southeast and Central-West regions may be constrained by local environmental conditions even where suitable hosts are present (Corrêa et al., 2024). Investigating the biological parameters and adaptive capacity of ERM under distinct thermal

and edaphic regimes is therefore essential for anticipating management needs in the emerging hubs of Brazilian viticulture and winemaking.

Finally, the detection of a genetically distinct lineage, represented here by populations from Chile, Canada, and Russia (GII), points to remarkable plasticity and the global establishment of a genotype that may be misidentified as ERM. This broad geographic range supports the hypothesis that this genotype is already widely distributed.

Author contributions

All authors contributed to the study conception and design. JMN performed experiments, data collection and writing the manuscript; LJ performed sample collection, supervision and writing; DN and NJF were the supervisors and reviewed the manuscript; DES, MS and FS contributed with colony maintenance and execution of experiments; GLS and JRP, analysis of data; RSM performed molecular data analysis, supervision and writing. All authors have read and approved the final manuscript.

Data availability

FASTA files for the *COI* and *ITS* sequences, as well as spreadsheets containing collection metadata and corresponding GenBank accession numbers for each sequence generated in this study, have been deposited in the Zenodo repository (DOI: https://doi.org/10.5281/zenodo.23039861).

The dataset is currently under embargo and will be made fully open access upon publication of this article. For peer-review purposes, the complete dataset can be accessed via the following private link: https://zenodo.org/records/23039861?preview=1&token=eyJhbGciOiJIUzUxMiIsImlhdCI6MTc5MDcwMTUzOCwiZXhwIjoxODE0MjI3MTk5fQ.eyJpZCI6Ijc5NjI2OGVmLTE5ZDMtNGU5Yy05MzVmLTFmMzhhMWUyM2Y4NiIsImRhdGEiOnt9LCJyYW5kb20iOiJmOGQ0M2M4YWYyNDQ0ZTBjNzhmNDczZjA0ZjFmYjNkMCJ9.Hfm7eR9cEfC3JGqFbK9xjiC6ZFnpAHKxjHRiG_dQQlIapelmVdvRbTfLPpt82QpXErNmeltfBkqkj7cIuEJaqA

Declarations

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Competing Interests

The authors have no competing interests to declare.

Ethics approval and consent to participate

Not applicable.

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