6-2015

Exceptionally High Levels of Genetic Diversity in Wheat Curl Mite (Acari: Eriophyidae) Populations from Turkey

W. Szydło
Adam Mickiewicz University, Poznań, Poland, wiktoria.szydlo@amu.edu.pl

Gary L. Hein
University of Nebraska-Lincoln, ghein1@unl.edu

E. Denizhan
Yüzüncü Yıl University, Turkey

A. Skoracka
Adam Mickiewicz University, Poznań, Poland

Published in *Journal of Economic Entomology* 108:4 (2015), pp. 2030–2039; doi: 10.1093/jee/tov180

Submitted March 29, 2015; accepted June 1, 2015; published online June 30, 2015.

Exceptionally High Levels of Genetic Diversity in Wheat Curl Mite (Acari: Eriophyidae) Populations from Turkey

W. Szydło,[1] G. Hein,[2] E. Denizhan,[3] and A. Skoracka[1]

1 Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland
2 Department of Entomology, University of Nebraska–Lincoln, P.O. Box 830933, Lincoln, NE 68583-0933, USA
3 Department of Plant Protection, Agricultural Faculty, Yüzüncü Yıl University, 65080 Van, Turkey

Corresponding author — W. Szydło, email wiktoria.szydlo@amu.edu.pl.

Abstract

Recent research on the wheat curl mite species complex has revealed extensive genetic diversity that has distinguished several genetic lineages infesting bread wheat (*Triticum aestivum* L.) and other cereals worldwide. Turkey is the historical region of wheat and barley (*Hordeum vulgare* L.) domestication and diversification. The close relationship between these grasses and the wheat curl mite provoked the question of the genetic diversity of the wheat curl mite in this region. The scope of the study was to investigate genetic differentiation within the wheat curl mite species complex on grasses in Turkey. Twenty-one wheat curl mite populations from 16 grass species from nine genera (*Agropyron* sp., *Aegilops* sp., *Bromus* sp., *Elymus* sp., *Eremopyrum* sp., *Hordeum* sp., *Poa* sp., *Secale* sp., and *Triticum* sp.) were sampled in eastern and southeastern Turkey for genetic analyses. Two molecular markers were amplified: the cytochrome oxidase subunit I coding region of mtDNA (COI) and the D2 region of 28S rDNA. Phylogenetic analyses revealed high genetic variation of the wheat curl mite in Turkey, primarily on *Bromus* and *Hordeum* spp., and exceptionally high diversity of populations associated with bread wheat. Three wheat-infesting wheat curl mite lineages known to occur on other continents of the world, including North and South America, Australia and Europe, were found in Turkey, and at least two new genetic lineages were discovered. These regions of Turkey exhibit rich wheat curl mite diversity on native grass species. The possible implications for further studies on the wheat curl mite are discussed.

Keywords: *Aceria tosichella*, genetics, variation, cryptic, complex

The wheat curl mite, *Aceria tosichella* (Keifer), is an invasive pest of increasing importance on cereal crops, primarily as a vector of plant viruses such as wheat streak mosaic virus (WSMV), wheat mosaic virus (WMoV; formerly a High Plains virus), and Triticum mosaic virus (TriMV; Slykhuis 1995; Seifers et al. 1997, 2009; Navia et al. 2013). The wheat curl mite has been observed on >80 grass species in North and South America, Africa, Australia, and Eurasia (Navia et al. 2013). However, recent research on the wheat curl mite revealed extensive genetic diversity and led to the conclusion that in fact wheat curl mite represents a cryptic species complex (Carew et al. 2009; Hein et al. 2012; Skoracka et al. 2012, 2013, 2014a; Miller et al. 2013). The existence of cryptic diversity (viz. morphologically similar but genetically different entities) within an economically important crop pest species, such as wheat curl mite, may have enormous implications for the control of the pest (Bickford et al. 2007). Species within a complex may differ in biological and ecological traits, for example, host range, effect on plant physiology, pesticide resistance, ability to transmit pathogens, and potential invasiveness (e.g., Perring 2001, Drés and Mallet 2002, Bickford et al. 2007, Guo et al. 2013, Frewin et al. 2014).

Indeed, experimental assays examining wheat curl mite host acceptance confirmed the ecological distinctiveness of several genotypes (some of them are presumed to be different species), but since this study has been limited to Poland, it is expected that the wheat curl mite complex is much more species rich (Skoracka et al. 2013). This study further showed that the host range strategy is uneven within the wheat curl mite complex, with some lineages exhibiting narrow or exclusive host ranges (e.g., MT-4 and MT-5 infesting smooth brome and tall oat-grass, respectively), whereas others are more generalist in nature, tending to infest a broader range of host plants. Nevertheless, a general host-related coevolutionary pattern has been suggested to exist among wheat curl mite genetic lineages (Miller et al. 2013). Three genetic wheat curl mite lineages have been identified as the most important economically, viz. MT-1, MT-7, and MT-8, because of their ability to infest bread wheat and common barley, as well as their worldwide occurrence, including North (MT-1, MT-8 only) and South America (MT-1 only), Australia, and Europe (Skoracka et al. 2014b). Long-distance dispersal abilities of eriophyoid mites are still to be investigated but may include aerial means of dispersal as well as dispersal on a

host, because their ability to disperse by walking is limited (Lindquist and Oldfield 1996, Sabelis and Bruin 1996, Michalska et al. 2010). The global spread of these wheat curl mite lineages was most likely associated with cultivated *Allioidae* plants (i.e., onion and garlic; Skoracka et al. 2014). Eriophyoid mites are haplodiploid and reproduce mostly by arrhenotokous parthenogenesis (Michalska et al. 2010, Miller et al. 2012), so just one founder female is able to start a new population.

Studies conducted on genetic variation of wheat curl mite populations to date have not addressed the genetic variation of wheat curl mite populations in regions where the cultivation of wheat and barley originated. The Turkish region, examined in this study, falls within or borders the historical region of the Fertile Crescent where the domestication of crops began (Lev-Yadun et al. 2000). This area is where einkorn wheat (*Triticum monococcum* L.), *Triticum araraticum* Jakubz., and common barley were domesticated (Heun et al. 1997, Smith 1998, Badr et al. 2000, Doebley et al. 2006). Furthermore, Turkey is currently a center of genetic diversity of wheat (Dvorak et al. 2011) and is the site of the natural origin of its progenitors. In addition to bread wheat and durum wheat (*Triticum durum* Desf.) cultivation, Turkey is still home to ancient agricultural species, including wild emmer (*Triticum dicoccum* L.) and einkorn wheat (Bardsley and Thomas 2005, Karagöz 2014).

Recently, the wheat curl mite has been recorded in Turkey from several host species, including bread wheat (Denizhan et al. 2013, Kiedrowicz et al. 2014), and all three cereal-associated lineages (viz. MT-1, MT-7, and MT-8) have been detected (Skoracka et al. 2014b). Taking into consideration the worldwide occurrence of wheat- and barley-infesting wheat curl mite lineages, Turkey and adjacent areas have been suggested to be the region of origin for the wheat curl mite species complex. A study on wheat curl mite diversity in this region would elucidate the relationships between host plants and wheat curl mite lineages and enable the testing of hypotheses on the possible patterns of the mite's early speciation and spread (Denizhan et al. 2013, Skoracka et al. 2014b).

The aim of this study was to explore the genetic diversity of different wheat curl mite host populations in Turkey. Specifically, we propose to: 1) assess the level of variation among the Turkish wheat curl mite populations inhabiting grasses for two genetic markers that are commonly used for eriophyoid mites, the cytochrome oxidase subunit I mitochondrial DNA coding region and the D2 region of 28S ribosomal DNA, and 2) verify the existence of wheat curl mite genetic lineages in Turkey that are present in other parts of the world.

Materials and Methods

Sampling. Sampling was conducted in eastern and southeastern Turkey from 2009 to 2011 as a part of a faunistic survey. The shoots of 16 grass (Poaceae) species from 10 genera were sampled, including *Agropyron* Gaertn., *Aegilops* L., *Bromus* L., *Dactylis* L., *Elymus* L., *Eremopyrum* (Ledeb.) Jaub. & Spach., *Hordeum* L., *Poa* L., *Secale* L., and *Triticum* L. The plants were examined under a stereomicroscope for the presence of eriophyoid mites. Mite specimens were collected and mounted on slides using a standard protocol (de Lillo et al. 2010) and then identified (Keifer 1969, Amrine et al. 2003). Specimens of *Aceria tosichella* Keifer as well as *Abacarus hystrix* (Nalepa), and *Abacarus longilobus* Skoracka were collected and preserved for further genetic analyses. A single sample for genetic analysis consisted of the mite specimens of one species collected from one plant shoot placed directly in extraction buffer (ATL buffer; Qiagen, Hilden, Germany). The samples were transferred to the Molecular Biology Techniques Laboratory at Adam Mickiewicz University (Poland) and stored at –20°C. The number of specimens per sample, hosts, dates and localities are shown in Table 1.

DNA Extraction, Amplification, and Sequencing. The number of mite specimens per grass sample used for genetic analysis varied from 5 to 25, and the samples only consisted of specimens originating from the same population. A nondestructive method of DNA extraction was applied, as described by Dabert et al. (2008), using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Post-digestion, specimen cuticles were transferred to 70% ethanol for further preparation and identification to eliminate the possibility of errors. A partial sequence of the cytochrome oxidase subunit I (COI) of mitochondrial DNA (mtDNA) was amplified via PCR using the degenerate primers bcdF01 and bcdR04 (Skoracka and Dabert 2010). A partial sequence of the ribosomal DNA (rDNA) ranging from ITS1 to the D2/D3 region of 28S rDNA was amplified with the primers D1D2fw2 (Skoracka and Dabert 2010) and 28Sr0990 (Mironov et al. 2012). PCR was conducted in a 10 ml reaction volume containing 5 ml of Type-it Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 50pM each primer and 4 ml of DNA template. The thermocycling profile consisted of one cycle of 5min at 95°C, followed by 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 50°C, and a 1 min extension for both the bcdF01/ bcdR04 and the D1D2fw2/28Sr0990 primer sets at 72°C, with a final step of 15 min at 72°C. The reaction products were diluted twofold and checked through electrophoresis on a 1% agarose gel with ethidium bromide. The COI amplification reaction products were directly sequenced in both directions with the same primers (bcdF01 and bcdR04). The amplified rDNA samples were enzymatically cleaned with a mixture of exonuclease I (Exo I) and Fast AP thermosensitive alkaline phosphatase (Thermo Fisher Scientific Inc., Waltham, MA) prior to sequencing. The samples were sequenced with the forward primer to obtain the D2 region of the 28S rDNA sequence: D1D2fw2 (Sonnenberg et al. 2007), Er28SF05 (5′-ACGAATCGGAGCCACGAAG- 3′) and reverse Er28SR05 (5′-TCGTCTAACTGGTTGCAGTG-3′). All sequencing was performed with BigDye Terminator v. 3.1 on an ABI Prism 3130XL or 3730 Analyzer (Applied Biosystems, Foster City, CA). The forward and reverse sequences were aligned and assembled with BioEdit v. 7 software (Hall 1999). Trace files were aligned and edited with MEGA5 (Tamura et al. 2011). All sequences have been deposited in NCBI GenBank under the accession numbers indicated in Table 1.

Table 1. Samples used in the study: eriophyoid mite species, host plant species, collection dates, collection locality coordinates, number of specimens collected in a single tube with buffer, sample codes, and GenBank accession numbers

Species	Host	Date	Lat-Long	No. of specimens per sample	Sample code	COI accession number	D2 accession number
Abacarus hystrix	*Elymus hispidus* (Opiz) Melderis subsp. Barbulatus	30-Aug-2009	38.450001, 43.400002	5; 10 (2 samples)	ahys.1	KC412843	KM280940
	Triticum aestivum L.	3-July-2010	39.56015, 44.099369	1	ahys.2	KC412844	–
Abacarus longilobus	*Bromus erectus* Huds.	3-July-2009	37.316669, 43.466671	8	alon.1	KC412846	KM280944
	Dactylis glomerata L.	24-July-2009	39.192089, 43.882778	20	alon.2	KC412845	–
Aceria tosichella	*Aegilops cylindrica* Host	4-June-2010	38.508209, 43.375439	10	wcm.AEG.1	KC412858	KM280947
	Agropyron cristatum (L.) Gaertn. Subsp. Inconum (Nab) Medler	27-June-2009	38.3256, 43.407619	5; 15; 15; 10 (4 samples)	wcm.AGR.1	KC412847	KM280942
	Arrhenatherum elatius (L.) Beauv. ex Presl & Presl	15-Sept-2009	52.4664, 16.9342	5	wcm.ARR.1	JQ248924[a]	JF920109[b]
	Bromus arvensis L.	3-July-2009	38.355499, 43.656898	3; 10; 7 (3 samples)	wcm.BRO.1	KC412848	KM280943
	Bromus tomentellus Boiss.	13-July-2009	38.316631, 43.800758	10	wcm.BRO.2	KC412850	KM280945
	Bromus cappadocius Boiss. Subsp. Cappadocius	30-July-2009	37.316669, 43.466671	5; 5 (2 samples)	wcm.BRO.3	KC412849	KM280946
	Bromus inermis Leyss.	30-Oct-2009	52.4675, 16.9086	4	wcm.BRO.4	JQ248922[a]	JQ918885[a]
	Elymus hispidus (Opiz) Melderis subsp. Barbulatus	10-Aug-2009	39.56015, 44.099369	12	wcm.ELY.1	KC412851	KM280941
	Elymus repens (L.) Gould	16-July-2013	51.547123, 15.010541	1	wcm.ELY.2	KP973941[c]	–
	Eremopyrum orientale (L.) Jaub. & Spach	25-June-2011	40.512086, 43.572825	25; 25 (2 samples)	wcm.ERE.1	KM280939	KM280956
	Hordeum giganteum (Vahl) Raspail	23-June-2010	38.333302, 43.4333	10	wcm.HOR.1	KC412853	KM280949
	Hordeum geniculatum All.	3-July-2010	38.333302, 43.4333	10	wcm.HOR.2	KC412854	KM280950
	Hordeum sp.	26-July-2010	38.176899, 43.949501	10	wcm.HOR.3	KC412855	–
	Hordeum violaceum Boiss. & Hohen.	16-July-2010	38.387798, 42.792198	10	wcm.HOR.4	KC412852	KM280957
	Hordeum sp.	9-July-2010	39.56015, 44.099369	10	wcm.HOR.5	KC412856	KM280959
	Hordeum murinum L.	1-Aug-2010	38.008041, 43.061039	10	wcm.HOR.6	KC412857	–
	Hordeum murinum L.	3-Dec-2010	52.4628, 16.9247	8	wcm.HOR.7	JQ248926[a]	JQ918888[a]
	Hordeum vulgare L.	28-June-2013	52.493953, 15.380120	1	wcm.HOR.8	KP973942[c]	–
	Poa bulbosa L.	26-July-2010	38.176899, 43.949501	10	wcm.POA.1	KC412860	KM280958
	Secale ciliatoglume (Boiss.) Grossh.	16-June-2010	39.028099, 43.36179	10	wcm.SEC.1	KC412859	KM280948
	Secale cereale L.	13-July-2013	51.937114, 16.024099	5	wcm.SEC.2	KP973943[c]	–
	Triticum aestivum L.	13-June-2011	39.028099, 43.36179	28	wcm.TRI.1	KC412861[d]	KM280951
	Triticum aestivum L.	18-June-2011	39.028099, 43.36179	25	wcm.TRI.2	KC412862	KM280952
	Triticum aestivum L.	13-June-2011	38.421223, 42.140324	25	wcm.TRI.3	KC412864	KM280953
	Triticum aestivum L.	19-June-2011	39.946251, 43.968361	25	wcm.TRI.4	KC412863	–
	Triticum aestivum L.	26-Oct-2011	40.534458, 43.109268	25	wcm.TRI.5	KC412865	KM280954
	Triticum aestivum L.	20-June-2011	39.56015, 44.099369	25	wcm.TRI.6	KC412866	KM280955
	Triticum aestivum L.	2-July-2009	52.043333, 16.767222	15	wcm.TRI.7	JF920077[b]	JF920097[b]
	Triticum aestivum L.	1996	41.235296, –103.002505	5	wcm.TRI.8	JX102055[e]	–

Sequences with the superscripts *a, b, c, d* and *e* were downloaded from GenBank:

a. Skoracka et al. 2013

b. Skoracka et al. 2012

c. W.S. and A.S., unpublished data

d. Skoracka et al. 2014b

e. Hein et al. 2012

All other sequences were obtained during the course of this study.

Alignment and Sequence Analyses. Twenty-one COI sequences and 18 rDNA sequences were obtained from various host populations of *Aceria tosichella* (wheat curl mite) from Turkey (Table 1). There were no differences among the COI sequences generated from mites originating from the same population, and single representatives of each wheat curl mite population were therefore used in the analyses. Four COI sequences and two rDNA sequences were obtained from *Abacarus hystrix* and *A. longilobus* from Turkey. Additional COI and D2 sequences from previously described wheat curl mite lineages, viz., MT-1, MT-2, MT-4, MT-5, MT-7, and MT-8 (Hein et al. 2012; Skoracka et al. 2012, 2013, 2014b), were downloaded from the NCBI GenBank database (Table 1).

A total of 33 COI sequences, aligned and edited with MEGA5 software to a length of 603 bp, were analyzed. A total of 24 rDNA sequences were aligned with MEGA5 software, and the alignment was trimmed to a length of 466 bp to cover the partial sequence of the D2 region of 28S rDNA. Two *A. hystrix* and *A. longilobus* sequences for each (mtDNA and rDNA) dataset served as outgroup taxa. All necessary alignment format conversions were performed with the web application Alignment Transformation Environment, ALTER (Glez-Peña et al. 2010).

For each dataset (COI and D2), neighbor-joining (NJ) trees with 1,000 bootstrap replicates were constructed with MEGA5 on the basis of the uncorrected distance (p-distance). The pair-wise p-distances and Kimura-2-parameter (K2P) distances between sequences and the overall mean distance of the sequence datasets were calculated with MEGA5 software, as were the between- and within-group p-distances (groups chosen *a posteriori* on the basis of the p-distance matrices). The uncorrected distance was chosen as a simple and reliable measure of sequence divergence for this study, whereas the K2P distance matrix was calculated as a comparable reference to previous research results (Skoracka et al. 2012, 2013). For maximum likelihood (ML) analysis using PhyML3.1 (Guindon et al. 2010), best-fit nucleotide substitution models for both alignments were chosen with jModeltest v. 2.1.3 (Darriba et al. 2012, Guindon and Gascuel 2003) according to the Akaiake information criterion (AIC), which were TPM2uf+I+G (p-inv = 0.55, G = 1.11) for the COI alignment and TrN+G (G = 0.3) for the D2 alignment. For the purpose of Bayesian inference (BI) analysis, the COI dataset was subdivided into three partitions based on the codon position (1st, 2nd, and 3rd), and the best-fit model scheme was chosen with Partition Finder v. 1.1.1 (Lanfear et al. 2012). The best-fit models under the Bayesian information criterion (BIC) for implementation in MrBayes 3.2.2 were SYM+G for the 1st codon position, F81 for the 2nd codon position and HKY+G for the 3rd codon position (gamma shape parameters set to be estimated by MrBayes 3.2.2). The best-fit model for BI analysis of the D2 alignment was K80+G (G = 0.32) according to the BIC, chosen with jModeltest v. 2.1.3.

ML analysis was performed separately for the datasets using PhML3.1 software. Bayesian inference analysis was performed for each dataset separately using MrBayes 3.2 (Ronquist et al. 2012), with two independent runs consisting of four chains each (3 heated and 1 cold), and data partitioning was applied. The analysis was performed until the average split deviation was below 0.01. The trace files were analyzed with Tracer v. 1.5 for effective sample size (Rambaut and Drummond 2009), and the 25% of trees obtained in the beginning of the analysis were discarded. Additionally, both datasets were concatenated into two-partitioned datasets of 24 sequences. Each concatenated sequence consisted of both the COI and D2 sequence from the same sample. The incongruence length difference test (ILD test; Farris et al. 1994, 1995) with 500 partition-homogeneity test replicates was performed with PAUP* 4.0b10 (Swofford 2003) to test the congruence of the data. Combined analysis for the concatenated alignment was performed with MrBayes 3.2 to obtain the consensus tree, with the nucleotide substitution models implemented as for the separate COI and D2 dataset Bayesian inference analyses.

Results

The mtDNA COI sequence dataset exhibited 240 variable sites, and the amino acid translation presented 23 variable sites. There was no evidence of indels or premature stop codons. The average nucleotide composition of the COI dataset was T = 44.4, C = 18.1, A = 21.9, and G = 15.7. The average transition/transversion ratio (R = transitional pair/transversional pair frequencies) was $R = 26$ (codon positions 1st, 2nd, and 3rd: $R = 13.40$, $R = 0.25$, and $R = 2$, respectively). The overall mean p-distance of the COI sequence dataset including the out-group sequences was 15.5% (SE = 0.9%), whereas the overall mean p-distance among the wheat curl mite sequences was 13.9% (SE = 0.9%). P-distance values for the wheat curl mite COI sequences ranged from 0.17 to 23.8%. The overall mean p-distance of the D2 sequence dataset including the out-group sequences was 5.11% (SE = 0.5%), and the overall mean p-distance was 4.03% (SE = 0.4%) for wheat curl mite sequences. P-distance values for the wheat curl mite D2 sequences ranged from 0 to 14.25%. The p-distances between the out-group and in-group were 21.0% (SE = 1.3%) for COI and 11.96% (SE = 1.4%) for D2. The matrices of pair-wise p-distances and K2P distances for the COI and D2 alignments are given in the Supplementary materials (Suppl. Tables 1–4).

The pair-wise p-distance matrices were examined for sequence pairs showing values of p-dist < 4% and p-dist < 0.4% for the COI and D2 sequence distance matrix, respectively. The values have been chosen on the basis of previous comprehensive analyses of the same DNA regions (e.g., Hebert et al. 2003, Sonnenberg et al. 2007) demonstrating the ranges of inter- and intraspecific distances between sequences of species pairs of various animals. Based on these matrices, sequences 18 from the COI alignment fell into seven groups, designated A, B, C, D, E, F, and G (Figure 1), exhibiting mean within-group p-distances ranging from 0.17% (SE = 0.15%;

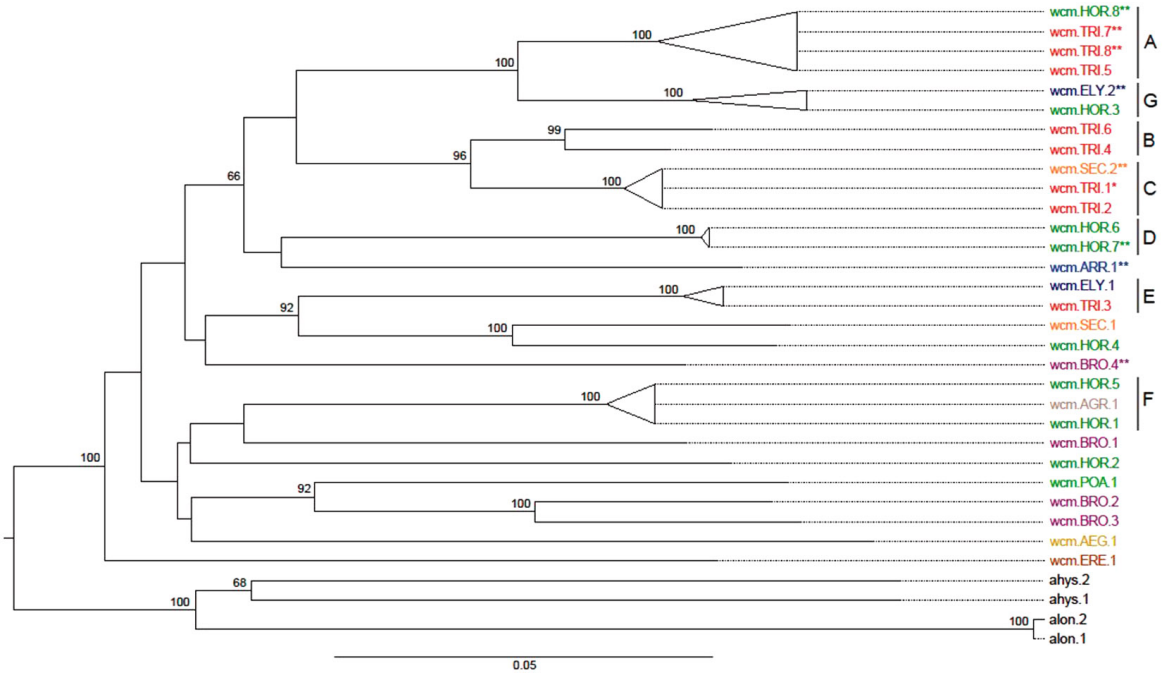

Figure 1. Neighbor-joining tree based on the p-distances of the mtDNA COI sequences dataset built with MEGA5. Values above the branches represent bootstrap-measured support (1,000 replicates) equal to or higher than 60. Groups indicated on the right of the tree tips were assigned a posteriori to the tree analysis based on pair-wise p-distance values lower than 4%. The sample codes are explained in Table 1. The colors of the code were assigned by the population host genus. * = sequence downloaded from GenBank from a population of Turkish origin; ** = sequence downloaded from GenBank from a population of non-Turkish origin (USA and Poland).

Table 2. Within group mean p-distances with standard error values (bootstrap = 1,000) given in percentages of the groups of COI mtDNA sequences (A–G) and the groups of D2 region of 28S rDNA sequences (a–d) used in the study

Group	COI							D2			
	A	B	C	D	E	F	G	a	b	c	d
Mean (%) p-distance	2.05	3.49	0.66	0.17	0.83	0.89	2.49	0	0.15	0.29	0.22
SE (%)	0.41	0.72	0.26	0.15	0.36	0.30	0.59	0	0.14	0.14	0.22

Group names are explained in the text.

group D) to 3.49% (SE = 0.7%; group B; Table 2). While 13 D2 sequences fell into four groups, designated a, b, c, and d (Figure 2), presenting mean within-group p-distances ranging from 0% (group a) to 0.22% (SE = 0.2%; group d; Table 2). Two of the groups were recovered for both datasets: group A corresponded to group a, and the F group corresponded to the b group.

Distance-based NJ analyses resulted in trees with a number of highly supported ancestral clades, while relationships among terminal nodes were poorly resolved (Figures 1 and 2). The COI tree topology confirmed the exceptionally high variation observed in the divergence values. The distance-based COI sequence groups shown in Figure 1 match the branching of the tree. There was no clear host association, as mite haplotypes from different host genera were paraphyletic. The sequences originating from mites infesting *Bromus* spp. and *Hordeum* spp. were scattered on tree tips without any trend, except for the following cases: 1) wcm.HOR.1 and wcm.HOR.5 clustered with wcm.AGR.1 for both COI (group F) and D2 (group b); 2) wcm.HOR.6 and wcm.HOR.7 (COI group D); and 3) wcm.BRO.2 and wcm.BRO.3 (D2 group d). The wcm.BRO.2 and wcm.BRO.3 sequences in the D2 region of 28S were similar (0.22%, SE = 0.2%; Figure 2); however, their COI sequences were as much as 6.67% distant (SE = 1%; Figure 1). The mtDNA COI sequences of the wheat-associated populations wcm.TRI.4 and wcm.TRI.6 clustered together (group B). The mtDNA COI sequences of the others wheat-associated populations fell into three groups (A, C, and E) but clustered with the sequence of wcm.HOR.8, wcm.SEC.2, and ELY.1, respectively (Figure 1).

The results of the ML analyses were concordant with the BI trees, and the latter results are shown with ML support values added (derived with the approximate likelihood ratio test; aLRT [Anisimova and Gascuel 2006]). The topology of the COI BI tree (Figure 3) was consistent with that obtained using the NJ method and supported the existence of groups A, D, E, F, and G.

The D2 BI tree (Figure 4) showed polytomy of four clusters and two stand-alone sequences (wcm.BRO.1 and wcm.BRO.4), with the sequences of the *Bromus*-, *Hordeum*- and wheat-associated wheat curl mite populations scattered among them without any clear host-related clustering. Groups a, b, and d were reflected in the BI tree, and the c group was found within a bigger polytomic clade that included wcm.SEC.1 and wcm.HOR.2.

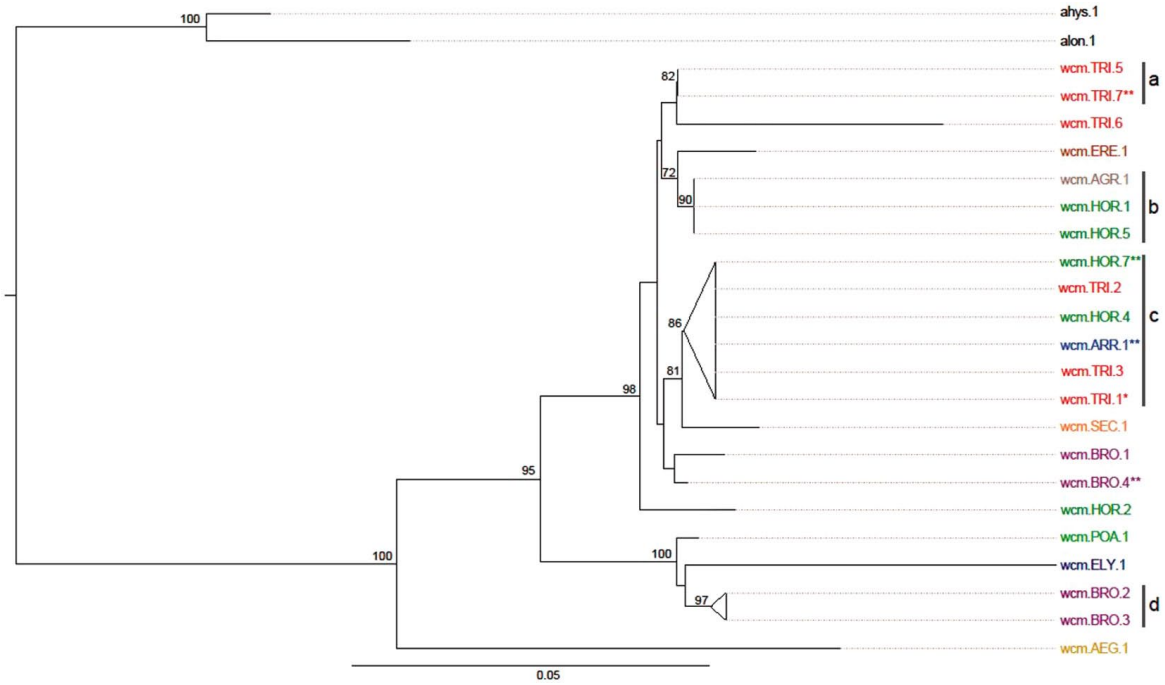

Figure 2. Neighbor-joining tree based on the p-distances of the D2 sequences of the 28S rDNA dataset built with MEGA5. Values above the branches represent bootstrap-measured support (1,000 replicates) equal to or higher than 60. Groups indicated on the right of the tree tips were assigned a posteriori to the tree analysis based on pair-wise p-distance values lower than 0.4%. The sample codes are explained in Table 1. The colors of the code were assigned by the population host genus. * = sequence downloaded from GenBank from a population of Turkish origin; ** = sequence downloaded from GenBank from a population of non-Turkish origin (USA and Poland).

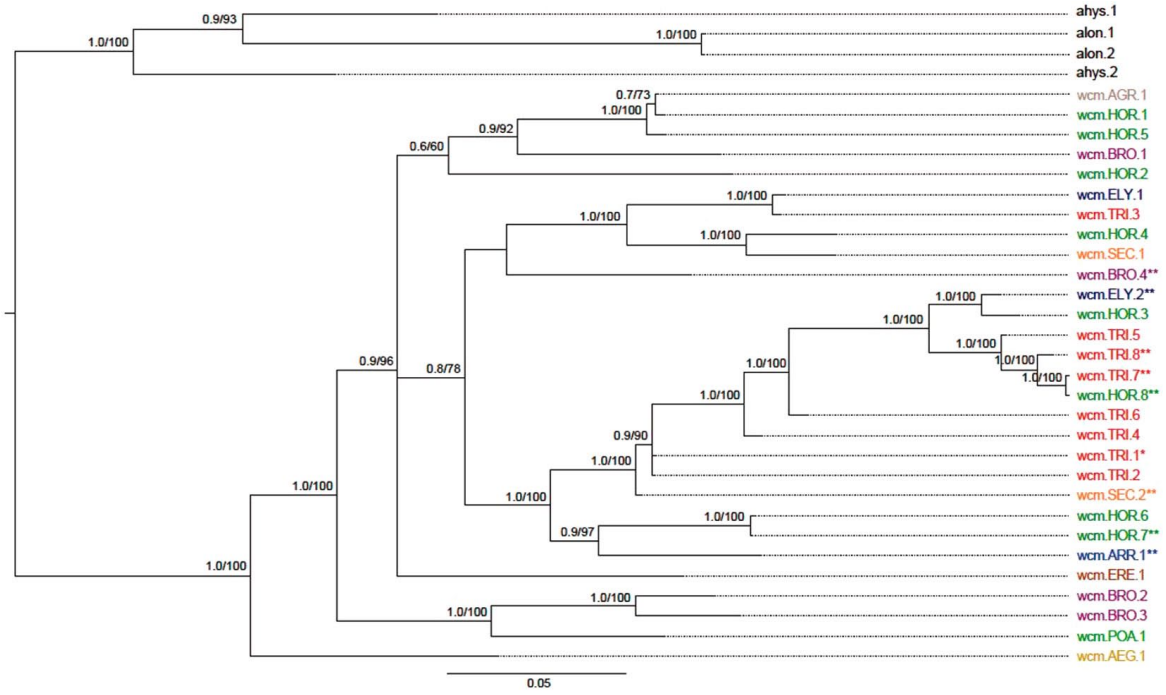

Figure 3. Bayesian tree of mtDNA COI sequences inferred with MrBayes 3.2. Values above the branches represent support values (aLRT from ML analysis and posterior probabilities of BI analysis) equal to or higher than 60 (0.6). The sample codes are explained in Table 1. The colors of the code were assigned by the population host genus. * = sequence downloaded from GenBank from a population of Turkish origin; ** = sequence downloaded from GenBank from a population of non-Turkish origin (USA and Poland).

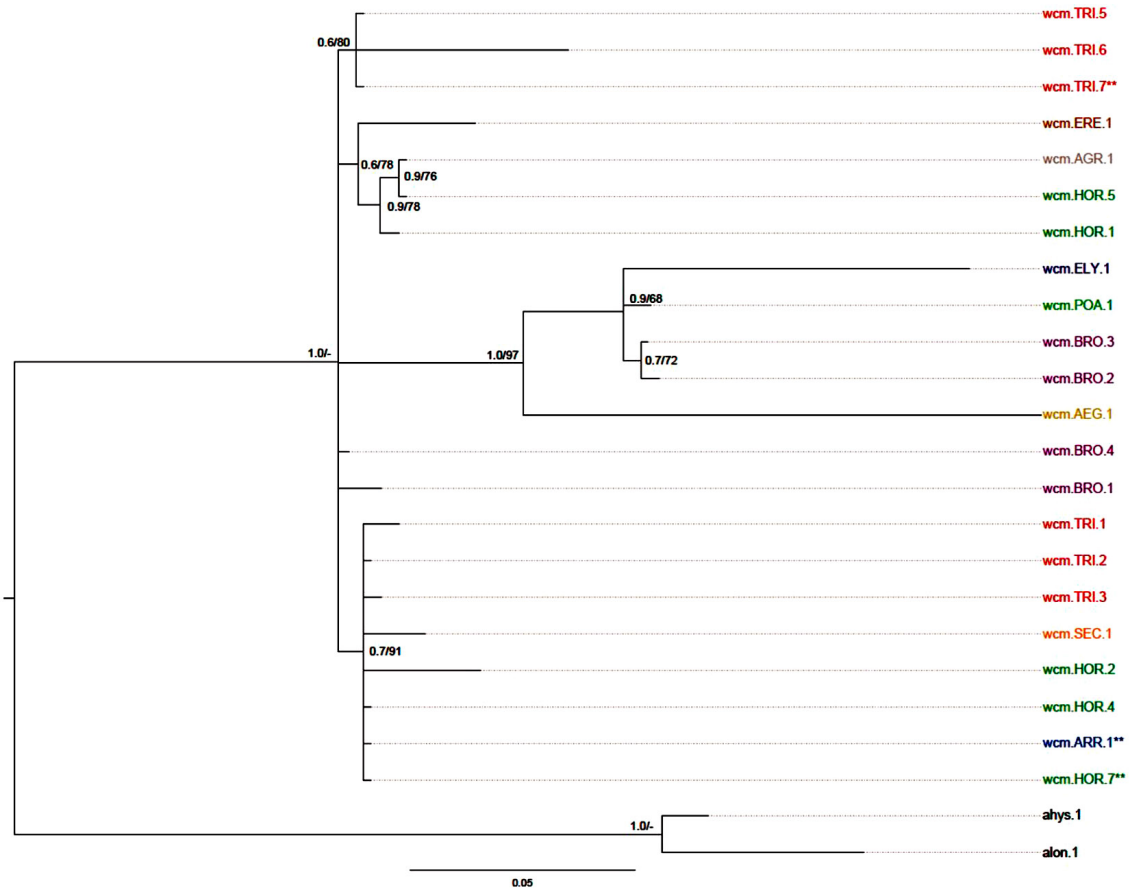

Figure 4. Bayesian tree of the D2 region of 28S rDNA sequences inferred with MrBayes 3.2. Values above the branches represent support values (aLRT from ML analysis and posterior probabilities of BI analysis) equal to or higher than 60 (0.6). The sample codes are explained in Table 1. The colors of the code were assigned by the population host genus. ** = sequence downloaded from GenBank from a population of non-Turkish origin (USA and Poland).

The COI and D2 sequence datasets were incongruent, as the ILD test P-value was less than $P = 0.05$ ($P < 0.05$). The tree inferred from the combined analysis represented an unreliable topology (tree not shown). But, it showed some similarities to the NJ and BI trees, with groups A and a, F and b, and group D being recovered in the topology.

Discussion

The results of this study, focusing on wheat curl mite populations from regions of Turkey, not only supported previous findings about genetic variation within wheat curl mite suggesting the existence of cryptic diversity, but above all uncovered much more diversity within the wheat curl mite species complex. This diversity matched the diversity observed on different continents (Skoracka et al. 2012, 2014b) and even exceeded that of the host species and mite pattern complexity. In addition to the lineages previously detected worldwide (Skoracka et al. 2013, 2014b), viz., MT-1 (in this study, in groups A and a), MT-2 (in group G), MT-7 (in groups D and c), and MT-8 (in groups C and c), we found several new genetic lineages in Turkey (Figures 1 and 2).

Among six Turkish wheat curl mite populations from *Hordeum* spp., only one (viz. wcm.HOR.6) was closely related to the globally present MT-7 lineage that attacks barley, *Hordeum vulgare* L., and bread wheat in Australia, and wall barley, *Hordeum murinum* L., in Poland (Skoracka et al. 2014b). The other five haplotypes were highly divergent from each other and apparently formed several putative lineages, with much uncertainty due to the incongruence of mitochondrial DNA-based and nuclear DNA-based phylogenies. Similarly, three haplotypes from *Bromus* spp. formed two new lines that were divergent from the previously detected in Poland (Skoracka et al. 2013; wcm.BRO.4, see Table 1 and Figures 1–4).

Most importantly, this study revealed great variation in the wheat curl mite populations from the agriculturally significant cereal grain bread wheat, with COI haplotypes falling into four distinct groups. Three wheat curl mite lineages were previously known to occur on bread wheat worldwide (Skoracka et al. 2014b). This study revealed three new wheat-associated haplotypes (viz., wcm.TRI.3, wcm.TRI.4, and wcm.TRI.6).

In addition, wheat curl mite populations from six other host plant genera (*Aegilops*, *Agropyron*, *Elymus*, *Eremopyrum*, *Poa*, and *Secale*) add to the wheat curl mite diversity

observed in Turkey. This high genetic variability exceeds the variation found in other studied areas, and provides evidence that the sampled area lies within the original distribution of the wheat curl mite complex, as previously suggested (Skoracka et al. 2014b). This has been demonstrated or suggested for other phytophagous pests and for invasive species (e.g., Karsten et al. 2013, Kirk et al. 2013, Zheng et al. 2013, Shi et al. 2014, Mastrangelo et al. 2014). Higher genetic variation in the native area of a species than in an invaded area might be a result of the bottlenecks and genetic drift experienced by invasive populations (e.g., Tsutsui et al. 2000, Sakai et al. 2001, de Barro and Ahmed 2011). The pattern of lower genetic diversity among wheat and barley wheat curl mite haplotypes worldwide compared to genetic divergence among wheat and barley wheat curl mite haplotypes in Turkey suggests that only some genotypes have successfully moved from Turkey to other areas around the world. This may be due to the differences between various wheat curl mite genotypes in their dispersal, adaptive, competitive, or survival ability. For example, the lineage MT-1 has been shown to survive on onion and garlic bulbs (Skoracka et al. 2014a), which might allow international movement of the mite. This issue should be experimentally tested in the future to show what biological characteristics of different wheat curl mite genotypes may influence their colonization and invasive potential.

Turkey is also a part of the native distribution of wheat curl mite hosts, including the Triticae tribe (*Aegilops* spp., *Elymus* spp., *Eremopyrum* spp., *Hordeum* spp., *Secale* spp., and *Triticum* spp.), many species of *Bromus*, and *Poa bulbosa*. This supports the existence of well-established mite and host plant relationships in this region. Some of these wheat curl mite host plants have spread worldwide as highly important crops, such as bread wheat and barley, or have invaded the North American continent, such as the close bread wheat relative *Aegilops cylindrica* (Donald and Ogg 1991) and *Poa bulbosa* (Novak and Welfley 1997). The founder populations responsible for the worldwide invasion of the wheat curl mite might have followed the spread of their hosts (and other hosts not mentioned here). Rabenstein et al. (2002) noted that the wheat streak mosaic virus (WSMV) and the wheat curl mite associated with hard red winter wheat might have been introduced from the Black Sea region, which was supported by the similarity of North American and Turkish WSMV isolates and the alleged late diversification of US strains of WSMV. These and our findings highlight the importance of understanding wheat curl mite species complex diversity in the area of its plausible origin for wheat curl mite and WSMV pathosystem management.

The host and mite coevolutionary pattern observed in this study was different from that reported by Miller et al. (2013), who revealed consistent host associations, with a single haplotype often dominating on a host plant species. Although it must be stated that Miller et al. (2013) used different genetic markers than other wheat curl mite studies applied (e.g., Hein et al. 2012; Skoracka et al. 2012, 2013, 2014b).

The observed incongruence of mtDNA and genomic DNA phylogenies may be explained by possible introgressive hybridizations of lineages or ancestral polymorphisms (Nowell et al. 2011, Stankowski and Johnson 2014, Dias and Carareto 2012). Hybridization may contribute to speciation through the formation of new hybrid taxa, whereas introgression of a few loci may promote adaptive divergence and so facilitate speciation (Abbott et al. 2013). Whether any of the above processes play a role in speciation of the wheat curl mite complex remains unclear, and further genetic investigations (e.g., including the whole genome sequencing) are required to explain this phenomenon. The incongruence between COI and D2 trees may question the reliability of these markers as barcodes for quick identification of wheat curl mite lineages. However, these findings confirmed earlier hypotheses on the very recent speciation within wheat curl mite complex and possible hybridization between lineages (e.g., Skoracka et al. 2012). Taking into account a higher rate of mutation in mtDNA compared to nuclear DNA and the potential earlier divergence in mtDNA than in nuclear genes after a recent speciation event (Piganeau and Eyre-Walker 2009), COI sequences make a better barcode than D2 for quarantine purposes.

Our study has expanded the known levels of diversity in wheat curl mite, and has led to the discovery of high diversity of the wheat curl mite species complex in Turkey. These results support the hypothesis regarding Middle East origin of the wheat curl mite. At least two lineages of wheat curl mite associated with wheat were found for the first time in Turkey, and the existence of additional lineages is possible. Turkey and the adjacent areas abound in wheat curl mite lineages associated with native grass species. The region may also support additional potentially invasive genotypes. Understanding the genetic and ecological variation of an invasive plant pest and virus vector is crucial for efficient management. For example, the phytophagous cryptic species complex of *Bemisia tabaci* Gannadius (Insecta: Hemiptera: Aleyrodidae) comprises several important lineages with diverse invasive abilities (de Barro and Ahmed 2011, Esterhuizen et al. 2013, Frewin et al. 2014). Furthermore, knowledge of wheat curl mite diversity on cultivated grasses and their noncultivated relatives or on host plant species associated with narrowly specialized wheat curl mite lineages may provide clues for the identification of genes that are responsible for resistance to wheat curl mite. This study elucidates the diversity of the wheat curl mite cryptic species complex in its presumed cradle of origin and signals the necessity for further research regarding the source and drivers of global wheat curl mite introduction events. This understanding is central to enhanced bio-security.

Acknowledgments — We wish to acknowledge Mirosława Dabert (Molecular Techniques Laboratory, Adam Mickiewicz University), who helped to design the Er28SF05 and Er28SR05 primers. The study was financed by the National Science Centre of Poland, grant DEC- 2011/01/N/NZ8/04540. DNA data of three wheat curl mite populations included here were financed by the National Science Centre of Poland, grant DEC-2011/03/B/NZ8/00129. We would also like

to thank the three anonymous reviewers from *Journal of Economic Entomology* for a critical reading of the manuscript and their valuable comments.

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Table S1. The pair-wise p-distances of the COI mtDNA sequences used in the study are presented on the lower-left side of the table, and relevant standard error values (bootstrap=1,000) are presented on the upper-right. All distances were calculated with MEGA5. The sample codes are explained in Table 1.

	ahys.1	ahys.2	alon.1	alon.2	wcm.AEG.1	wcm.AGR.1	wcm.ARR.1	wcm.BRO.1	wcm.BRO.2	wcm.BRO.3	wcm.BRO.4	wcm.ELY.1	wcm.ELY.2	wcm.ERE.1	wcm.HOR.1	wcm.HOR.2	wcm.HOR.3	wcm.HOR.4	wcm.HOR.5	wcm.HOR.6	wcm.HOR.7	wcm.HOR.8	wcm.POA.1	wcm.SEC.1	wcm.SEC.2	wcm.TRI.1	wcm.TRI.2	wcm.TRI.3	wcm.TRI.4	wcm.TRI.5	wcm.TRI.6	wcm.TRI.7	wcm.TRI.8
ahys.1		1.62	1.72	1.70	1.81	1.73	1.68	1.69	1.71	1.78	1.76	1.72	1.88	1.64	1.74	1.73	1.89	1.71	1.73	1.68	1.67	1.70	1.63	1.82	1.68	1.72	1.73	1.73	1.76	1.78	1.77	1.70	1.75
ahys.2	17.17		1.73	1.74	1.76	1.60	1.78	1.81	1.71	1.76	1.78	1.71	1.68	1.73	1.58	1.63	1.69	1.73	1.58	1.75	1.76	1.73	1.69	1.71	1.73	1.70	1.71	1.71	1.77	1.70	1.79	1.73	1.76
alon.1	21.17	19.83		0.16	1.85	1.71	1.73	1.78	1.73	1.79	1.81	1.72	1.75	1.79	1.71	1.77	1.76	1.72	1.74	1.70	1.68	1.74	1.73	1.72	1.70	1.69	1.69	1.74	1.73	1.74	1.74	1.74	1.71
alon.2	21.00	20.00	0.17		1.85	1.71	1.73	1.78	1.74	1.80	1.82	1.74	1.76	1.80	1.71	1.78	1.77	1.73	1.73	1.70	1.68	1.75	1.73	1.73	1.69	1.69	1.69	1.76	1.73	1.75	1.74	1.75	1.72
wcm.AEG.1	21.33	19.50	23.67	23.83		1.53	1.54	1.56	1.55	1.49	1.55	1.55	1.60	1.61	1.52	1.49	1.63	1.48	1.58	1.54	1.54	1.76	1.53	1.51	1.58	1.57	1.58	1.54	1.62	1.69	1.62	1.76	1.77
wcm.AGR.1	20.17	18.83	21.67	21.50	15.00		1.45	1.33	1.46	1.39	1.33	1.47	1.41	1.43	0.21	1.41	1.43	1.42	0.38	1.45	1.45	1.46	1.49	1.52	1.39	1.37	1.39	1.45	1.38	1.44	1.45	1.46	1.46
wcm.ARR.1	18.00	20.83	21.83	21.67	18.67	13.83		1.47	1.56	1.50	1.36	1.46	1.47	1.51	1.46	1.46	1.51	1.35	1.46	1.36	1.35	1.49	1.54	1.38	1.17	1.17	1.19	1.41	1.32	1.41	1.32	1.49	1.44
wcm.BRO.1	19.50	20.83	21.33	21.50	16.33	11.17	14.67		1.50	1.51	1.37	1.50	1.49	1.50	1.35	1.39	1.52	1.49	1.33	1.54	1.54	1.48	1.39	1.59	1.44	1.44	1.45	1.48	1.44	1.46	1.47	1.48	1.52
wcm.BRO.2	20.17	19.17	21.33	21.50	15.83	15.17	16.33	14.00		1.02	1.50	1.57	1.50	1.57	1.48	1.58	1.57	1.42	1.46	1.56	1.56	1.56	1.29	1.49	1.55	1.55	1.56	1.58	1.55	1.53	1.61	1.56	1.57
wcm.BRO.3	20.33	20.83	22.33	22.50	16.17	13.67	16.33	14.33	6.67		1.43	1.60	1.56	1.59	1.40	1.52	1.62	1.50	1.41	1.55	1.55	1.61	1.29	1.56	1.49	1.50	1.52	1.58	1.55	1.56	1.56	1.61	1.59
wcm.BRO.4	20.50	21.33	23.83	24.00	17.50	12.67	14.17	13.67	15.50	15.33		1.43	1.37	1.40	1.35	1.39	1.45	1.35	1.32	1.34	1.32	1.43	1.40	1.44	1.35	1.38	1.40	1.37	1.42	1.40	1.42	1.43	1.43
wcm.ELY.1	20.67	20.67	20.67	20.83	17.17	14.33	15.83	15.33	16.50	17.50	13.67		1.41	1.58	1.46	1.39	1.47	1.33	1.48	1.39	1.40	1.47	1.51	1.34	1.39	1.36	1.37	0.35	1.30	1.39	1.37	1.47	1.48
wcm.ELY.2	22.33	19.33	22.33	22.50	18.83	14.00	14.67	15.17	16.17	17.17	13.33	14.33		1.54	1.41	1.49	0.63	1.54	1.40	1.40	1.40	1.04	1.46	1.48	1.33	1.34	1.35	1.40	1.09	0.96	1.10	1.04	0.98
wcm.ERE.1	19.33	19.50	22.00	22.17	18.50	14.17	16.33	16.00	17.50	18.50	15.00	17.33	16.33		1.44	1.52	1.60	1.49	1.44	1.44	1.43	1.57	1.48	1.53	1.42	1.42	1.43	1.55	1.42	1.55	1.48	1.57	1.54
wcm.HOR.1	20.50	18.83	22.00	21.83	15.00	0.33	14.17	11.50	15.50	14.00	13.00	14.33	14.33	14.50		1.41	1.41	1.40	0.44	1.45	1.46	1.47	1.50	1.51	1.40	1.36	1.37	1.45	1.37	1.43	1.45	1.47	1.47
wcm.HOR.2	20.67	20.67	21.83	22.00	16.17	13.67	15.83	13.33	16.33	15.83	14.50	14.00	14.33	17.17	13.67		1.42	1.55	1.41	1.55	1.56	1.52	1.50	1.61	1.47	1.44	1.45	1.36	1.47	1.46	1.44	1.52	1.52
wcm.HOR.3	23.17	19.33	22.33	22.50	19.50	14.17	15.67	15.67	17.33	17.50	14.83	15.17	2.50	17.17	14.17	13.83		1.60	1.42	1.50	1.50	1.09	1.54	1.57	1.37	1.34	1.35	1.48	1.10	1.04	1.13	1.09	1.05
wcm.HOR.4	20.83	20.33	22.67	22.83	17.00	14.33	14.50	15.33	15.50	17.50	13.83	12.67	17.00	15.33	14.33	17.83	18.17		1.44	1.55	1.56	1.61	1.50	1.01	1.39	1.37	1.38	1.27	1.42	1.54	1.48	1.61	1.63
wcm.HOR.5	20.17	18.50	21.83	21.67	16.00	1.00	14.17	11.17	15.17	14.00	12.50	14.50	14.17	14.33	1.33	13.67	14.33	14.50		1.49	1.48	1.45	1.50	1.56	1.38	1.37	1.39	1.45	1.38	1.44	1.45	1.45	1.45
wcm.HOR.6	19.83	21.17	20.67	20.83	17.17	13.33	11.83	15.17	16.17	16.67	13.33	13.67	13.83	15.33	13.33	16.00	14.83	15.83	14.17		0.15	1.40	1.49	1.54	1.27	1.25	1.25	1.40	1.26	1.34	1.35	1.40	1.41
wcm.HOR.7	19.67	21.33	20.50	20.67	17.33	13.17	11.67	15.00	16.00	16.50	13.17	13.83	13.67	15.17	13.50	16.17	15.00	16.00	14.00	0.17		1.39	1.49	1.55	1.25	1.26	1.27	1.41	1.28	1.35	1.34	1.39	1.40
wcm.HOR.8	20.00	20.67	22.33	22.50	20.33	14.17	15.83	14.67	16.33	17.00	13.33	14.83	6.83	16.33	14.50	15.33	8.00	17.33	14.33	13.67	13.50		1.47	1.60	1.44	1.46	1.47	1.45	1.22	0.65	1.31	0.00	0.54
wcm.POA.1	19.83	19.67	21.83	22.00	17.83	16.00	16.33	13.67	12.17	12.83	16.17	17.83	16.50	16.17	16.33	14.83	17.50	18.00	16.00	15.67	15.50	16.50		1.51	1.47	1.46	1.47	1.46	1.46	1.39	1.47	1.47	1.48
wcm.SEC.1	22.50	20.33	23.33	23.50	16.17	15.83	14.67	16.33	15.50	17.50	13.83	11.67	15.50	15.83	15.83	17.50	17.33	7.17	16.33	16.17	16.33	17.17	17.33		1.42	1.39	1.40	1.33	1.43	1.54	1.50	1.60	1.60
wcm.SEC.2	18.17	20.00	20.83	21.00	18.33	12.83	10.17	14.83	16.00	15.50	11.83	13.83	13.67	14.17	13.17	15.67	13.83	14.50	13.17	10.17	10.00	13.83	15.33	15.17		0.37	0.33	1.33	0.87	1.36	0.90	1.44	1.43
wcm.TRI.1	18.17	19.50	21.00	21.17	18.17	12.83	10.50	14.83	16.00	15.83	11.83	13.50	13.50	14.17	12.83	15.00	13.33	14.50	12.83	10.17	10.33	13.83	15.33	14.83	1.00		0.17	1.31	0.80	1.35	0.92	1.46	1.45
wcm.TRI.2	18.33	19.67	20.83	21.00	18.33	13.00	10.67	15.00	16.17	16.00	12.00	13.67	13.67	14.33	13.00	15.17	13.50	14.67	13.00	10.33	10.50	14.00	15.50	15.00	0.83	0.17		1.31	0.82	1.36	0.91	1.47	1.46
wcm.TRI.3	20.50	20.83	21.00	21.17	17.17	14.17	15.17	15.00	16.33	17.00	12.83	0.83	14.17	17.17	14.17	13.50	15.33	11.83	14.00	13.83	14.00	15.00	17.00	11.50	13.33	13.00	13.17		1.30	1.38	1.33	1.45	1.47
wcm.TRI.4	19.67	20.50	21.00	21.17	19.83	13.00	12.33	14.50	16.33	16.67	12.50	12.17	9.00	14.67	13.00	14.83	9.33	15.17	13.17	10.67	10.83	10.50	15.50	15.00	5.33	4.67	4.83	12.00		1.05	0.72	1.22	1.18
wcm.TRI.5	20.67	19.67	22.33	22.50	19.50	13.83	13.83	14.17	16.17	16.83	13.17	13.83	5.67	16.33	13.83	13.67	6.67	16.17	14.00	13.00	13.17	3.00	15.33	16.33	12.50	12.17	12.33	13.67	8.00		1.16	0.65	0.57
wcm.TRI.6	19.67	20.50	21.50	21.67	19.83	14.17	12.50	16.00	17.33	16.67	13.00	13.50	8.33	15.33	14.50	15.17	8.83	16.17	14.33	11.67	11.50	11.17	16.17	16.50	5.67	6.00	5.83	13.00	3.33	8.67		1.31	1.23
wcm.TRI.7	20.00	20.67	22.33	22.50	20.33	14.17	15.83	14.67	16.33	17.00	13.33	14.83	6.83	16.33	14.50	15.33	8.00	17.33	14.33	13.67	13.50	0.00	16.50	17.17	13.83	13.83	14.00	15.00	10.50	3.00	11.17		0.54
wcm.TRI.8	20.83	21.17	22.00	22.17	20.50	14.83	14.67	14.83	16.67	17.33	13.67	15.83	6.33	16.67	15.17	15.00	7.50	17.67	15.00	13.67	13.50	2.00	16.50	17.17	14.00	14.00	14.17	15.67	10.17	2.33	10.50	2.00	

Table S2. The pair-wise p-distances of the D2 region of 28S rDNA sequences used in the study are presented on the lower-left side of the table and relevant standard error values (bootstrap=1,000) are presented on the upper-right. All distances were calculated with MEGA5. The sample codes are explained in Table 1.

	ahys.1	alon.1	wcm.AEG.1	wcm.AGR.1	wcm.ARR.1	wcm.BRO.1	wcm.BRO.2	wcm.BRO.3	wcm.BRO.4	wcm.ELY.1	wcm.ERE.1	wcm.HOR.1	wcm.HOR.2	wcm.HOR.4	wcm.HOR.5	wcm.HOR.7	wcm.POA.1	wcm.SEC.1	wcm.TRI.1	wcm.TRI.2	wcm.TRI.3	wcm.TRI.5	wcm.TRI.6	wcm.TRI.7
ahys.1		0.86	1.55	1.40	1.39	1.37	1.54	1.52	1.38	1.73	1.44	1.40	1.38	1.39	1.40	1.39	1.52	1.41	1.40	1.39	1.40	1.38	1.54	1.38
alon.1	3.79		1.60	1.55	1.53	1.52	1.58	1.57	1.54	1.77	1.57	1.55	1.56	1.53	1.55	1.53	1.53	1.54	1.54	1.53	1.54	1.52	1.61	1.52
wcm.AEG.1	12.69	14.25		1.48	1.47	1.44	1.39	1.38	1.46	1.59	1.51	1.48	1.44	1.47	1.48	1.47	1.43	1.52	1.46	1.47	1.48	1.45	1.64	1.45
wcm.AGR.1	10.24	12.47	10.69		0.41	0.55	0.96	0.93	0.37	1.29	0.53	0.00	0.65	0.41	0.00	0.41	0.90	0.62	0.51	0.41	0.46	0.38	0.93	0.38
wcm.ARR.1	10.24	12.47	10.91	0.89		0.48	0.93	0.90	0.36	1.29	0.61	0.41	0.60	0.00	0.41	0.00	0.88	0.48	0.29	0.00	0.22	0.35	0.92	0.35
wcm.BRO.1	9.80	12.47	10.02	1.56	1.11		0.98	0.96	0.42	1.34	0.71	0.55	0.74	0.48	0.55	0.48	0.93	0.66	0.56	0.48	0.52	0.51	1.00	0.51
wcm.BRO.2	12.25	13.36	9.58	4.90	4.68	5.12		0.22	0.94	1.03	1.00	0.96	1.04	0.93	0.96	0.93	0.42	1.00	0.96	0.93	0.95	0.95	1.23	0.95
wcm.BRO.3	12.03	13.14	9.35	4.68	4.45	4.90	0.22		0.91	1.01	0.98	0.93	1.01	0.90	0.93	0.90	0.37	0.98	0.94	0.90	0.93	0.92	1.22	0.92
wcm.BRO.4	9.80	12.47	10.47	0.67	0.67	0.89	4.68	4.45		1.30	0.59	0.37	0.68	0.36	0.37	0.36	0.89	0.58	0.47	0.36	0.42	0.30	0.92	0.30
wcm.ELY.1	16.70	17.82	14.25	9.58	9.35	9.80	5.79	5.57	9.35		1.36	1.29	1.35	1.29	1.29	1.29	1.04	1.32	1.31	1.29	1.30	1.30	1.43	1.30
wcm.ERE.1	10.69	12.92	11.36	1.34	1.78	2.45	5.35	5.12	1.56	10.24		0.53	0.78	0.61	0.53	0.61	0.95	0.77	0.68	0.61	0.64	0.58	1.03	0.58
wcm.HOR.1	10.24	12.47	10.69	0.00	0.89	1.56	4.90	4.68	0.67	9.58	1.34		0.65	0.41	0.00	0.41	0.90	0.62	0.51	0.41	0.46	0.38	0.93	0.38
wcm.HOR.2	10.47	12.92	10.47	2.23	1.78	2.90	5.57	5.35	2.45	10.24	3.12	2.23		0.60	0.65	0.60	1.03	0.78	0.66	0.60	0.62	0.62	1.05	0.62
wcm.HOR.4	10.24	12.47	10.91	0.89	0.00	1.11	4.68	4.45	0.67	9.35	1.78	0.89	1.78		0.41	0.00	0.88	0.48	0.29	0.00	0.22	0.35	0.92	0.35
wcm.HOR.5	10.24	12.47	10.69	0.00	0.89	1.56	4.90	4.68	0.67	9.58	1.34	0.00	2.23	0.89		0.41	0.90	0.62	0.51	0.41	0.46	0.38	0.93	0.38
wcm.HOR.7	10.24	12.47	10.91	0.89	0.00	1.11	4.68	4.45	0.67	9.35	1.78	0.89	1.78	0.00	0.89		0.88	0.48	0.29	0.00	0.22	0.35	0.92	0.35
wcm.POA.1	12.03	12.69	10.02	4.45	4.23	4.68	0.89	0.67	4.23	5.79	4.90	4.45	5.57	4.23	4.45	4.23		0.96	0.91	0.88	0.90	0.89	1.18	0.89
wcm.SEC.1	10.69	12.92	11.80	2.00	1.11	2.23	5.57	5.35	1.78	10.24	2.90	2.00	2.90	1.11	2.00	1.11	5.12		0.55	0.48	0.52	0.58	0.99	0.58
wcm.TRI.1	10.69	12.92	11.36	1.34	0.45	1.56	5.12	4.90	1.11	9.58	2.23	1.34	2.23	0.45	1.34	0.45	4.68	1.56		0.29	0.37	0.46	0.96	0.46
wcm.TRI.2	10.24	12.47	10.91	0.89	0.00	1.11	4.68	4.45	0.67	9.35	1.78	0.89	1.78	0.00	0.89	0.00	4.23	1.11	0.45		0.22	0.35	0.92	0.35
wcm.TRI.3	10.47	12.69	11.14	1.11	0.22	1.34	4.90	4.68	0.89	9.58	2.00	1.11	2.00	0.22	1.11	0.22	4.45	1.34	0.67	0.22		0.41	0.93	0.41
wcm.TRI.5	10.02	12.03	10.47	0.67	0.67	1.34	4.68	4.45	0.45	9.35	1.56	0.67	2.00	0.67	0.67	0.67	4.23	1.78	1.11	0.67	0.89		0.87	0.00
wcm.TRI.6	12.92	14.25	13.81	4.45	4.45	5.12	8.24	8.02	4.23	11.80	5.35	4.45	5.79	4.45	4.45	4.45	7.80	5.35	4.90	4.45	4.68	3.79		0.87
wcm.TRI.7	10.02	12.03	10.47	0.67	0.67	1.34	4.68	4.45	0.45	9.35	1.56	0.67	2.00	0.67	0.67	0.67	4.23	1.78	1.11	0.67	0.89	0.00	3.79	

Table S3. Pair-wise Kimura 2-parameter (K2P) distances of the COI mtDNA sequences used in the study are presented in the lower-left side of the table and relevant standard error values (bootstrap=1000) are presented in the upper-right. All distances were calculated with MEGA5. Sample codes are explained in a Table 1.

	ahys.1	ahys.2	alon.1	alon.2	wcm.AEG.1	wcm.AGR.1	wcm.ARR.1	wcm.BRO.1	wcm.BRO.2	wcm.BRO.3	wcm.BRO.4	wcm.ELY.1	wcm.ELY.2	wcm.ERE.1	wcm.HOR.1	wcm.HOR.2	wcm.HOR.3	wcm.HOR.4	wcm.HOR.5	wcm.HOR.6	wcm.HOR.7	wcm.HOR.8	wcm.POA.1	wcm.SEC.1	wcm.SEC.2	wcm.TRI.1	wcm.TRI.2	wcm.TRI.3	wcm.TRI.4	wcm.TRI.5	wcm.TRI.6	wcm.TRI.7	wcm.TRI.8
ahys.1		2.1	2.7	2.7	2.6	2.5	2.2	2.3	2.4	2.5	2.5	2.6	2.7	2.4	2.5	2.5	2.8	2.5	2.5	2.5	2.5	2.5	2.5	2.7	2.3	2.3	2.3	2.6	2.5	2.5	2.5	2.5	2.5
ahys.2	20.1		2.5	2.5	2.4	2.2	2.5	2.5	2.2	2.4	2.5	2.4	2.3	2.3	2.2	2.4	2.3	2.4	2.1	2.4	2.4	2.4	2.3	2.4	2.4	2.4	2.4	2.5	2.5	2.4	2.4	2.4	2.5
alon.1	25.7	23.4		0.2	2.9	2.4	2.5	2.5	2.5	2.6	2.7	2.3	2.6	2.5	2.4	2.6	2.6	2.6	2.4	2.4	2.4	2.5	2.7	2.6	2.4	2.4	2.4	2.4	2.4	2.6	2.5	2.5	2.5
alon.2	25.5	23.6	0.2		2.9	2.4	2.5	2.5	2.5	2.6	2.7	2.3	2.6	2.6	2.4	2.6	2.6	2.6	2.4	2.4	2.4	2.5	2.7	2.7	2.4	2.4	2.4	2.4	2.4	2.6	2.5	2.5	2.5
wcm.AEG.1	25.8	23.2	29.0	29.2		1.8	2.3	2.1	1.9	1.9	2.1	2.2	2.3	2.3	1.8	2.0	2.3	2.1	1.9	2.1	2.2	2.5	2.1	2.0	2.3	2.3	2.3	2.2	2.5	2.4	2.5	2.5	2.5
wcm.AGR.1	24.0	21.9	25.9	25.6	16.9		1.9	1.6	1.9	1.7	1.6	1.9	1.8	1.8	0.2	1.8	1.8	1.8	0.4	1.8	1.8	1.8	2.0	2.0	1.7	1.7	1.7	1.8	1.7	1.8	1.8	1.8	1.9
wcm.ARR.1	21.0	24.9	26.3	26.0	22.0	15.6		1.9	2.1	2.1	1.9	2.2	2.0	2.2	1.9	2.0	2.1	1.9	2.0	1.7	1.7	2.1	2.2	2.0	1.6	1.6	1.6	2.1	1.8	1.9	1.8	2.1	1.9
wcm.BRO.1	23.0	24.9	25.5	25.7	18.8	12.3	16.7		1.8	1.9	1.8	2.1	2.0	2.0	1.6	1.7	2.0	2.0	1.6	2.1	2.0	1.9	1.9	2.1	2.0	1.9	2.0	2.0	1.9	1.8	2.1	1.9	1.9
wcm.BRO.2	23.8	22.4	25.4	25.6	18.0	17.2	18.7	15.7		1.1	1.9	2.1	2.0	2.1	1.9	2.0	2.1	1.9	1.8	2.0	2.0	2.0	1.7	2.0	2.1	2.0	2.1	2.1	2.0	2.0	2.2	2.0	2.1
wcm.BRO.3	24.1	24.9	26.9	27.2	18.4	15.2	18.7	16.1	7.1		1.8	2.2	2.1	2.3	1.7	1.9	2.1	2.2	1.7	2.0	2.0	2.1	1.7	2.2	2.0	2.0	2.0	2.2	2.1	2.1	2.1	2.1	2.1
wcm.BRO.4	24.5	25.7	29.2	29.5	20.3	14.1	16.2	15.3	17.6	17.3		1.8	1.8	1.9	1.7	1.8	1.9	1.8	1.6	1.7	1.7	1.7	2.0	1.8	1.6	1.6	1.6	1.7	1.7	1.7	1.8	1.7	1.8
wcm.ELY.1	24.7	24.6	24.5	24.7	19.9	16.2	18.3	17.4	18.9	20.2	15.4		2.0	2.2	1.9	1.8	2.1	1.8	1.8	1.9	2.0	1.9	2.3	1.6	1.9	1.9	1.9	0.4	1.7	1.8	1.8	1.9	2.1
wcm.ELY.2	27.2	22.7	26.8	27.1	22.3	15.8	16.8	17.3	18.5	19.8	15.1	16.4		2.1	1.8	1.8	0.6	2.2	1.8	1.8	1.8	1.2	2.1	2.1	1.9	1.9	1.9	1.9	1.3	1.0	1.3	1.2	1.1
wcm.ERE.1	22.7	22.9	26.5	26.7	21.6	16.0	18.8	18.3	20.2	21.6	17.0	20.3	18.9		1.9	2.2	2.2	2.0	1.9	2.0	2.0	2.2	2.1	2.0	1.9	1.9	1.9	2.2	2.0	2.2	2.1	2.2	2.2
wcm.HOR.1	24.5	21.9	26.3	26.1	16.9	0.3	16.0	12.6	17.6	15.6	14.5	16.2	16.2	16.4		1.8	1.8	1.8	0.5	1.8	1.8	1.8	2.0	2.0	1.7	1.6	1.7	1.8	1.7	1.8	1.9	1.8	1.9
wcm.HOR.2	24.6	24.6	26.2	26.4	18.5	15.3	18.1	14.8	18.6	17.9	16.4	15.7	16.1	19.8	15.3		1.7	2.2	1.7	2.1	2.1	2.0	1.9	2.2	2.0	1.9	1.9	1.7	1.9	1.8	1.9	2.0	1.9
wcm.HOR.3	28.4	22.7	26.8	27.1	23.2	15.9	18.0	17.9	20.1	20.3	17.0	17.5	2.6	20.0	15.9	15.5		2.4	1.8	1.9	1.9	1.3	2.2	2.3	1.9	1.8	1.8	2.1	1.3	1.1	1.3	1.3	1.2
wcm.HOR.4	24.8	24.2	27.4	27.7	19.6	16.2	16.5	17.4	17.6	20.3	15.6	14.3	20.0	17.5	16.2	20.8	21.7		1.8	2.0	2.1	2.2	2.3	1.2	2.0	2.0	2.0	1.7	2.0	2.1	2.1	2.2	2.3
wcm.HOR.5	24.0	21.4	26.1	25.9	18.2	1.0	16.0	12.2	17.1	15.6	13.9	16.4	16.0	16.2	1.3	15.3	16.1	16.4		1.8	1.8	1.8	2.0	2.1	1.7	1.6	1.7	1.8	1.7	1.8	1.8	1.8	1.9
wcm.HOR.6	23.6	25.4	24.6	24.8	19.9	14.9	13.3	17.3	18.4	19.1	15.0	15.4	15.6	17.5	14.9	18.3	16.9	18.3	16.0		0.2	1.7	2.1	2.2	1.5	1.5	1.6	2.0	1.6	1.7	1.7	1.7	1.7
wcm.HOR.7	23.4	25.6	24.4	24.6	20.1	14.7	13.1	17.1	18.2	18.9	14.8	15.6	15.4	17.3	15.1	18.5	17.1	18.5	15.8	0.2		1.7	2.1	2.2	1.5	1.6	1.6	2.0	1.6	1.7	1.7	1.7	1.7
wcm.HOR.8	23.6	24.7	26.8	27.1	24.4	16.0	18.3	16.6	18.7	19.6	15.0	17.0	7.3	18.9	16.4	17.4	8.6	20.4	16.2	15.3	15.1		2.1	2.3	1.9	1.8	1.9	1.9	1.5	0.7	1.6	0.0	0.6
wcm.POA.1	23.4	23.2	26.4	26.6	20.7	18.3	18.8	15.3	13.5	14.3	18.5	20.8	19.0	18.5	18.8	16.7	20.3	21.1	18.3	17.9	17.7	18.9		2.3	2.0	2.0	2.0	2.2	2.1	2.0	2.1	2.1	2.1
wcm.SEC.1	27.3	24.2	28.4	28.7	18.4	18.1	16.7	18.7	17.6	20.3	15.6	13.0	17.9	18.2	18.1	20.3	20.5	7.7	18.8	18.7	18.9	20.2	20.1		2.0	2.0	2.0	1.6	2.0	2.2	2.2	2.3	2.2
wcm.SEC.2	21.3	23.7	24.8	25.1	21.6	14.3	11.2	16.9	18.2	17.6	13.1	15.6	15.5	16.0	14.7	17.9	15.6	16.5	14.7	11.2	11.0	15.7	17.5	17.3		0.4	0.4	1.8	1.0	1.8	1.1	1.9	1.9
wcm.TRI.1	21.2	22.9	25.0	25.3	21.3	14.3	11.6	16.8	18.2	18.0	13.1	15.2	15.2	15.9	14.3	17.0	15.0	16.5	14.2	11.2	11.4	15.6	17.4	16.9	1.0		0.2	1.8	0.9	1.7	1.1	1.8	1.9
wcm.TRI.2	21.5	23.2	24.8	25.0	21.6	14.5	11.8	17.1	18.4	18.2	13.3	15.4	15.4	16.1	14.5	17.2	15.2	16.7	14.5	11.4	11.6	15.9	17.7	17.1	0.8	0.2		1.8	1.0	1.8	1.1	1.9	1.9
wcm.TRI.3	24.5	24.9	25.0	25.2	19.9	15.9	17.4	17.0	18.6	19.5	14.3	0.8	16.1	20.1	15.9	15.1	17.7	13.3	15.7	15.6	15.8	17.2	19.6	12.8	15.0	14.5	14.7		1.7	1.8	1.8	1.9	2.0
wcm.TRI.4	23.3	24.4	25.0	25.2	23.7	14.4	13.8	16.4	18.6	19.1	13.9	13.5	9.7	16.6	14.4	16.7	10.1	17.4	14.6	11.7	11.9	11.5	17.7	17.1	5.6	4.9	5.0	13.3		1.2	0.8	1.5	1.4
wcm.TRI.5	24.6	23.3	26.8	27.1	23.3	15.5	15.6	15.9	18.5	19.4	14.8	15.6	5.9	18.8	15.5	15.3	7.1	18.8	15.7	14.6	14.8	3.1	17.4	19.1	14.0	13.5	13.8	15.4	8.6		1.4	0.7	0.6
wcm.TRI.6	23.4	24.4	25.8	26.0	23.7	16.0	14.1	18.4	20.0	19.1	14.6	15.1	8.9	17.5	16.4	17.2	9.5	18.7	16.2	13.0	12.8	12.3	18.6	19.1	6.0	6.3	6.1	14.5	3.4	9.3		1.6	1.5
wcm.TRI.7	23.6	24.7	26.8	27.1	24.4	16.0	18.3	16.6	18.7	19.6	15.0	17.0	7.3	18.9	16.4	17.4	8.6	20.4	16.2	15.3	15.1	0.0	18.9	20.2	15.7	15.6	15.9	17.2	11.5	3.1	12.3		0.6
wcm.TRI.8	24.8	25.4	26.4	26.6	24.7	16.8	16.7	16.8	19.1	20.0	15.4	18.3	6.7	19.2	17.2	17.0	8.0	20.8	17.0	15.4	15.2	2.0	18.9	20.1	15.9	15.8	16.1	18.0	11.1	2.4	11.5	2.0	

Table S4. The pair-wise Kimura 2-parameter (K2P) distances of the D2 region of the 28S rDNA sequences used in the study are presented on the lower-left side of the table, and relevant standard error values (bootstrap=1,000) are presented on the upper-right. All distances were calculated with MEGA5. The sample codes are explained in Table 1.

	ahys.1	alon.1	wcm.AEG.1	wcm.AGR.1	wcm.ARR.1	wcm.BRO.1	wcm.BRO.2	wcm.BRO.3	wcm.BRO.4	wcm.ELY.1	wcm.ERE.1	wcm.HOR.1	wcm.HOR.2	wcm.HOR.4	wcm.HOR.5	wcm.HOR.7	wcm.POA.1	wcm.SEC.1	wcm.TRI.1	wcm.TRI.2	wcm.TRI.3	wcm.TRI.5	wcm.TRI.6	wcm.TRI.7
ahys.1		0.91	1.83	1.65	1.62	1.59	1.88	1.85	1.59	2.22	1.70	1.65	1.61	1.62	1.65	1.62	1.85	1.70	1.66	1.62	1.65	1.61	1.86	1.61
alon.1	3.89		1.98	1.82	1.81	1.82	1.95	1.93	1.82	2.31	1.86	1.82	1.85	1.81	1.82	1.81	1.85	1.86	1.84	1.81	1.82	1.76	1.93	1.76
wcm.AEG.1	13.95	15.88		1.75	1.76	1.69	1.62	1.59	1.73	1.99	1.80	1.75	1.69	1.76	1.75	1.76	1.67	1.85	1.79	1.76	1.78	1.72	2.09	1.72
wcm.AGR.1	11.08	13.75	11.69		0.43	0.60	1.06	1.03	0.38	1.49	0.54	0.00	0.68	0.43	0.00	0.43	0.99	0.63	0.53	0.43	0.48	0.39	0.98	0.39
wcm.ARR.1	11.08	13.75	11.97	0.90		0.49	1.02	0.99	0.36	1.46	0.60	0.43	0.60	0.00	0.43	0.00	0.96	0.49	0.31	0.00	0.22	0.37	0.99	0.37
wcm.BRO.1	10.55	13.75	10.88	1.58	1.13		1.09	1.06	0.43	1.55	0.72	0.60	0.81	0.49	0.60	0.49	1.02	0.71	0.59	0.49	0.54	0.54	1.09	0.54
wcm.BRO.2	13.49	14.82	10.39	5.12	4.88	5.37		0.21	1.03	1.13	1.10	1.06	1.14	1.02	1.06	1.02	0.45	1.11	1.07	1.02	1.05	1.03	1.43	1.03
wcm.BRO.3	13.21	14.54	10.13	4.88	4.64	5.12	0.22		1.01	1.11	1.08	1.03	1.11	0.99	1.03	0.99	0.39	1.08	1.05	0.99	1.02	1.00	1.42	1.00
wcm.BRO.4	10.55	13.75	11.42	0.67	0.67	0.90	4.88	4.64		1.49	0.57	0.38	0.70	0.36	0.38	0.36	0.97	0.58	0.48	0.36	0.43	0.30	0.98	0.30
wcm.ELY.1	18.98	20.42	15.98	10.27	10.01	10.54	6.03	5.79	10.01		1.57	1.49	1.55	1.46	1.49	1.46	1.14	1.51	1.49	1.46	1.48	1.49	1.75	1.49
wcm.ERE.1	11.58	14.26	12.46	1.35	1.81	2.50	5.59	5.34	1.58	11.04		0.54	0.80	0.60	0.54	0.60	1.04	0.76	0.68	0.60	0.64	0.56	1.11	0.56
wcm.HOR.1	11.08	13.75	11.69	0.00	0.90	1.58	5.12	4.88	0.67	10.27	1.35		0.68	0.43	0.00	0.43	0.99	0.63	0.53	0.43	0.48	0.39	0.98	0.39
wcm.HOR.2	11.34	14.29	11.40	2.27	1.81	2.98	5.85	5.61	2.51	11.05	3.20	2.27		0.60	0.68	0.60	1.13	0.80	0.69	0.60	0.63	0.62	1.11	0.62
wcm.HOR.4	11.08	13.75	11.97	0.90	0.00	1.13	4.88	4.64	0.67	10.01	1.81	0.90	1.81		0.43	0.00	0.96	0.49	0.31	0.00	0.22	0.37	0.99	0.37
wcm.HOR.5	11.08	13.75	11.69	0.00	0.90	1.58	5.12	4.88	0.67	10.27	1.35	0.00	2.27	0.90		0.43	0.99	0.63	0.53	0.43	0.48	0.39	0.98	0.39
wcm.HOR.7	11.08	13.75	11.97	0.90	0.00	1.13	4.88	4.64	0.67	10.01	1.81	0.90	1.81	0.00	0.90		0.96	0.49	0.31	0.00	0.22	0.37	0.99	0.37
wcm.POA.1	13.21	13.98	10.93	4.64	4.39	4.88	0.90	0.67	4.39	6.03	5.10	4.64	5.85	4.39	4.64	4.39		1.04	1.02	0.96	0.98	0.97	1.38	0.97
wcm.SEC.1	11.60	14.29	13.01	2.04	1.12	2.27	5.83	5.59	1.81	11.04	2.96	2.04	2.96	1.12	2.04	1.12	5.34		0.57	0.49	0.53	0.59	1.07	0.59
wcm.TRI.1	11.60	14.29	12.50	1.35	0.45	1.58	5.36	5.11	1.12	10.27	2.26	1.35	2.27	0.45	1.35	0.45	4.87	1.58		0.31	0.38	0.50	1.04	0.50
wcm.TRI.2	11.08	13.75	11.97	0.90	0.00	1.13	4.88	4.64	0.67	10.01	1.81	0.90	1.81	0.00	0.90	0.00	4.39	1.12	0.45		0.22	0.37	0.99	0.37
wcm.TRI.3	11.34	14.01	12.22	1.12	0.22	1.35	5.11	4.87	0.90	10.27	2.03	1.12	2.04	0.22	1.12	0.22	4.63	1.35	0.67	0.22		0.43	1.01	0.43
wcm.TRI.5	10.82	13.20	11.42	0.67	0.67	1.35	4.88	4.64	0.45	10.01	1.58	0.67	2.04	0.67	0.67	0.67	4.39	1.81	1.12	0.67	0.90		0.92	0.00
wcm.TRI.6	14.24	15.93	15.44	4.60	4.60	5.32	8.80	8.55	4.36	12.87	5.55	4.60	6.05	4.60	4.60	4.60	8.29	5.56	5.08	4.60	4.84	3.89		0.92
wcm.TRI.7	10.82	13.20	11.42	0.67	0.67	1.35	4.88	4.64	0.45	10.01	1.58	0.67	2.04	0.67	0.67	0.67	4.39	1.81	1.12	0.67	0.90	0.00	3.89	