



# Article First Field Record of the Tropical Red-Banded Thrips Selenothrips rubrocinctus (Thripidae: Panchaetothripinae) in Europe

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**Abstract:** Red-banded thrips *Selenothrips rubrocinctus* is a polyphagous pest widely distributed in tropical and subtropical regions. Various fruit trees such as cacao, cashew, mango, avocado, and guava in certain habitats are attacked. This is the first report of the spontaneous occurrence of outdoor populations of this species of thrips in Europe. This species has been found in northern Italy on *Liquidambar styraciflua*, *Carpinus betulus*, and other ornamental forest species in urban areas. Barcode sequences of the Italian specimens were obtained.

**Keywords:** Thysanoptera; Panchaetothripinae; cacao thrips; tropical pest; invasive species; Italy; molecular characterization; DNA barcoding; forest trees

### 1. Introduction

*Selenothrips rubrocinctus* (Giard, 1901) (Thysanoptera: Thripidae) is a tropical and subtropical species, probably originating from northern South America [1]. It was reported from Brazil, Guiana, Ecuador, Peru, Suriname, Venezuela, but also from North and Central America (Florida, Mexico, Costa Rica, Honduras, Panama), from Asia (China, Malaya, Philippine Islands, Taiwan), Central Africa, South Africa, Australasia, and Pacific Islands [2,3]. It is the only species belonging to the genus.

First described as cacao (or cocoa) thrips due to considerable damages caused on cacao plants *Theobroma cacao* L. in Guadeloupe (Antilles, Caribbean), it is also known as red-banded thrips, due to the presence of two characteristic bright red bands around the abdomen at nymphal stage [4].

Selenothrips rubrocinctus is widely polyphagous, attacking a variety of tropical fruit trees and ornamental shrubs such as avocado (*Persea americana* Mill.), mango (*Mangifera indica* L.), cashew (*Anacardium occidentale* L.), papaya (*Carica papaya* L.) guava (*Psidium guajava* L.), mangosteen (*Garcinia mangostana* L.), rambutan (*Nephelium lappaceum* L.). *S. rubrocinctus* is a sporadic, but potentially serious, pest on avocado fruit in South Africa [5]. It can become a severe pest on young mango plants [6,7], but Grove et al. [2] report low population density in the orchards and good natural biological control in South Africa. In Brazil, it has been detected also on lychee (*Litchi chinensis* Sonn.), guanandi (*Calophyllum brasiliense* Cambess.) and sweetgum (*Liquidambar styraciflua* L.) [8,9]. Moreover, it is acknowledged as a pest on Brazilian vineyards (*Vitis vinifera* L.), which can reduce fruit quality and, in case of intense



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). infestation, cause partial or total defoliation of the plants [10–12]. It is reported as a serious pest on Tung-oil trees (*Vernicia fordii* (Hemsl.) Airy Shaw) in southern China, where about eight overlapping generations can occur from mid-May to October, with a peak in late September [13]. Concerning ornamental plants, it has been reported in China on *Viburnum odoratissimum* Ker Gawl. and *Rhododendron simsii* Planch., causing serious damage to those species in urban green spaces of Hangzhou (Zhejiang Province) [14]. Etienne et al. [15] collected adult specimens and pupae from *Rosa* sp. leaves in Guadeloupe and Martinique, but did not record any feeding damage, which is instead documented by Walter et al. [16]. Reyes [17] includes *Quercus* trees, as well as *Solanum lycopersicum* L., in the list of plants associated to *S. rubrocinctus* in the Philippines.

In 2012, it was reported for the first time in Iran, with specimens collected from a forest in the Kordestan Province [18].

Adult thrips are dark brown to black in colour, with dark wings, folded along the back when at rest and a reddish pigmentation is visible in the first three and last abdominal segments. They are about 1.2 mm long. Immature stages are about 1 mm in length, bright yellow to orange, and with the first three and last abdominal segments bright red [3].

Eggs are inserted in the lower surface of the leaves and are covered with a drop of fluid that forms a black, circular shelter when dry. After hatching, two nymphal stages follow. They are able to move and feed, sucking the content out of leaf cells. A drop of faeces can often be seen at the end of the abdomen of actively-feeding nymphs. When released, faeces dry on the surface of the leaf, resulting in black spots. Resting stages (a pre-pupa and a pupa) appear after the two nymphal stages and feeding activity ceases, until adults emerge. In Florida, the life cycle is completed in about three weeks and several generations per year occur [3].

*Selenothrips rubrocinctus* has been intercepted several times in international trade by European plant health authorities. From 1980 until 1995, the Netherlands Plant Protection Organization found it on *Codiaeum* plants imported from Sri Lanka and Togo [19–21]. Afterwards, it was detected on *Garcinia* sp. from Thailand in 2005, on *Codiaeum* sp. from Surinam in 2011, and on *Codiaeum* sp. and *Liriope* sp. from Costa Rica in 2011, 2013, and 2014 [22]. The thrips were found by the plant health authorities of England and Wales during quarantine inspections on imported plant material: on *Codiaeum* sp. from Sri Lanka in 1995, on non-specified fruits from Indonesia in 1999, on *Psidium guajava* from Jamaica in 2003, and on *Garcinia* sp. from Thailand in 2005 [23]. In 2009, it was found in a glasshouse in Poland on *Codiaeum variegatum* (L.) A.Juss. plants imported from Sri Lanka [24]. According to Kobro [25], *S. rubrocinctus* larvae were intercepted in Sola, Norway, but on which plant is not reported in the publication.

In July 2015 an infestation of thrips sp. was noticed by Andrea Wojnar (Doctor of forestry, freelance) on some *Liquidambar styraciflua* and *Koelreuteria paniculata* Laxm. trees in the city centre of Palazzo Pignano, Cremona province, Italy (45°23′29.0"N 9°34′03.7"E). The trees were already in a state of vegetative stress due to water deficit and showed early leaf drop. Green areas in other adjacent municipalities were checked in order to assess the spread of the phenomenon. The general habitus of the specimens, especially of the immature stages, suggested that it could be *S. rubrocinctus*.

In this paper, we report the first occurrence of *S. rubrocinctus* in northern Italy on different forest plant species. This is the first official field record of this thrips in Europe, according to the available literature and official reports in the European Plant Protection Organization (EPPO Global Database). Until now, the known distribution of this insect was limited to tropical and subtropical regions. Also, the genetic barcoding sequences of the Italian specimens are provided.

#### 2. Materials and Methods

#### 2.1. Collection of Specimens and Morphological Identification

From 2016 to 2020, unofficial survey activities were carried out which confirmed the establishment of the thrips species in the area. Beside *Liquidambar styraciflua* and

*Koelreuteria paniculata*, other trees species were found to be attacked. Photographs of infestation were taken with a Nikon Coolpix B700 camera (vertical and horizontal resolution of 300 pixels, width of 3264 pixels, height of 2448 pixels) and an Olympus SP560UZ camera (vertical and horizontal resolution of 72 pixels, width of 3264 pixels, height of 2448 pixels). Adult and larval specimens were collected in different municipalities in the provinces of Cremona and Lodi (Lombardia Region) on June 24, 2019. Entire leaves were taken from lower branches of *Acer campestre* L., *Carpinus betulus* L., *Koelreuteria paniculata, Liquidambar styraciflua* and *Parrotia persica* (DC.) C.A.Mey. and put in plastic bags which were then sealed. In the laboratory, after freezing at -80 °C for 1 h, thrips were transferred, using an entomological brush, into Eppendorf tubes filled with 95% ethanol, for storage. Specimens from Trescore Cremasco (Cremona) were available from a previous sample carried out in 2016. The mounting procedure used was according to Mound & Kibby [26] in Hoyers mountant for larvae and Canada Balsam for adults. Pre-pupae and pupae were collected from infested leaves, but they were not included in the identification process.

Identification keys of Wilson [27] and Mirab-Balou et al. [28] were used. The specimens were compared with NRC reference material sampled in Vietnam and from Dutch import inspections of material originating from Togo, Sri Lanka, Thailand, Costa Rica and Surinam. The genus *Selenothrips* is identified with the key given by Wilson [27]. Two species were originally placed in this genus, but one of these, *S. glabratus* Priesner, 1927, was afterwards moved to another taxon (*Xestothrips*) due to the smooth head and pronotum [1]. A smooth head and pronotum is a rare character within the Panchaetothripinae. The species *S. rubrocinctus*, shows some differences in morphology between populations in body size and forewing length [27]. These characters were measured from females of different origin, together with the length/width ratio of antennal segments. The neotype (Guadeloupe) was compared on these characters with a large and a small female from Italy and with collection material of Netherlands Food and Consumer Product Safety Authority.

Males are rarely sampled and were not compared with collection material in detail. Nevertheless, the position and size of three pairs of spines on tergite IX was compared with collection material (Cape Verde Islands and Surinam) from the Senckenberg Institut at Frankfurt am Main, Germany.

Identified specimens were deposited in the NRC collection (Wageningen, Netherlands) and at the Department of Agriculture, Mediterranean University of Reggio Calabria (Reggio Calabria, Italy).

#### 2.2. DNA extraction and Amplification

Specimens from the same sample as above were submitted for molecular analysis in order to obtain barcode sequences. Total genomic DNA was extracted from 25 single specimens (5 first instar larvae, 20 adults) in a non-destructive way, using either the Qiagen Blood & Tissue Kit (Qiagen, Hilden, Germany) or a Chelex Extraction Protocol by Vono et al. [29]. Protocols were slightly modified. Polymerase chain reaction (PCR) was used to amplify a fragment of the mitochondrial cytochrome c oxidase gene, subunit 1 (COI), using the primers reported in Table 1.

Table 1. List of primers used to amplify the cytochrome c oxidase I (COI) region.

Primer Name	Sequence (5'-3')	Source	Reference
LCO-1490 (fw)	GGTCAACAAATCATAAAGATATTGG	Folmer et al.,1994	[30]
LCO1490puc-t1 (fw)	caggaaacagctatgaccTTTCAACWAATCATAAAGATATTGG	EPPO, 2016	[31]
LCO1490Hem1-t1 (fw)	caggaaacagctatgaccTTTCAACTAAYCATAARGATATYGG	EPPO, 2016	[31]
HCO-2198 (rv)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al.,1994	[30]
HCO2198puc-t1 (rv)	tgtaaaacgacggccagtTAAACTTCWGGRTGWCCAAARAATCA	EPPO, 2016	[31]
HCO2198Hem1-t1 (rv)	tgtaaaacgacggccagtTAAACYTCDGGATGBCCAAARAATCA	EPPO, 2016	[31]
HCO2198Hem2-t1 (rv)	tgtaaaacgacggccagtTAAACYTCAGGATGACCAAAAAAYCA	EPPO, 2016	[31]

The PCR mix had a total volume of 25  $\mu$ L and contained 2.5 mM MgCl<sub>2</sub>, primer cocktail at a concentration of 200 nM, 0.1 mM dNTPs, 2  $\mu$ L of genomic DNA and 1 unit of TaqDNA

polymerase (GoTaq G2 Flexi, Promega, Madison, WI). The amplification profile consisted of one step of 3 min at 94 °C, 5 cycles of 30 s at 94 °C, 30 s at 45 °C and 1 min at 72 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 51 °C and 1 min at 72 °C, with a final elongation step of 10 min at 72 °C. The concentration of the DNA samples and their purity was determined by Nanodrop, and the PCR products were checked on a 1.2% agarose gel stained with GelRED® (Biotium, Fremont, CA, USA). All amplicons produced a single band and were cleaned using the ExoSAP protocol. Amplification products were sent for Sanger sequencing (GATC Biotech, Köln, Germany and BMR Genomics, Italy). Forward and reverse sequences were aligned in BioEdit version 7.2.5 [32], manually trimmed and were virtually translated into the corresponding amino acid chain to detect frame-shift mutations and stop codons using EMBOSS Transeq [33]. Edited sequences were searched against the BOLD Systems v. 4 and GenBank databases in order to determine the closest match and confirm the identification based on morphological characters. The sequences obtained were submitted to the GenBank database under the accession numbers LR812089, MT926000, MT939151and represent the first sequences of S. rubrocinctus specimens found in Europe and occurring in an outdoor environment. Voucher specimens were deposited in the collection at ANSES Laboratoire de la santé des Végétaux, Unité d'Entomologie et Plantes invasives (Montferrier-sur-Lez, France) and at the Department of Agriculture, Mediterranean University of Reggio Calabria (Reggio Calabria, Italy). To display graphically COI genetic differences among S. rubrocinctus specimens a phylogenetic tree was produced. Using Partition Finder version 2.1.1, Oxford, United Kinkgdom [34], the best-fitted model was identified, and then cluster analysis was carried out using Neighbour-Joining method [35] via MEGA: Molecular Evolutionary Genetics Analysis version 7.0 for Bigger Dataset [36]. Bootstrap analysis was performed based on 1000 resampling. The COI sequence of Caliothrips indicus (Bagnall, 1913) (KX622205) was used as an outgroup.

#### 3. Results

#### 3.1. Morphological Characteristics

*Selenothrips rubrocinctus* specimens were identified from all samples (Table 2; Figures A1–A3 in Appendix A). Among the other diagnostic characters that allow the identification of this species, the metascutellar triangle with two strong median setae was observed (Figure A2B in Appendix A). Ten males and 38 females were identified, resulting in a male/female ratio of 0.26. The common presence of larvae of *S. rubrocinctus* indicate active reproduction on the sampled host plants.

**Table 2.** Records of *Selenothrips rubrocinctus* sampled on 24 June 2019 in Italy (Lombardia). Samples from Trescore Cremasco were collected in 2016.

Sample Nr.	Location	Lat., Long.	Host Plant	Material Sampled
5683090	Bagnolo Cremasco (1)	45°21′39.1″ N 9°36′31.4″ E	Carpinus betulus	2♀, 1♂, 2 larvae II, 4 larvae I
5683146	Bagnolo Cremasco (2)	45°21′40.2″ N 9°36′32.2″ E	Carpinus betulus	4♀, 1♂
5683082	Cremosano	45°22′49.5″ N 9°39′13.8″ E	Koelreuteria paniculata	3♀, 2♂, 4 larvae II, 6 larvae I
5683121	Crespiatica (1)	45°21′37.7″ N 9°34′22.9″ E	Acer campestre	19, 4 larvae I
5683103 *	Crespiatica (2)	45°21′37.6″ N 9°34′22.4″ E	Liquidambar styraciflua	7♀, 2 larvae II, 6 larvae I
5683154	Pandino	45°24′05.3″ N 9°33′34.4″ E	Liquidambar styraciflua	5♀, 10 larvae I
5683138 *	Palazzo Pignano (Scannabue)	45°23′13.9″ N 9°35′29.2″ E	Parrotia persica	1♂, 3 larvae I
26082016	Trescore Cremasco	45°24′06.1″ N 9°37′40.2″ E	Carpinus betulus	16♀, 5♂, 10 larvae I

\* Additionally in 5683103 and 5683138 respectively 29and 19Haplothrips kurdjumovi Karny.

No differences in the position and size of three pairs of spines on tergite IX (Figure A2A in Appendix A) could be detected when the Italian male specimens were compared with collection material (Cape Verde Islands and Surinam) from the Senckenberg Institut.

Some female specimens from Italy displayed a relatively high body size, forewing length, and length/width ratio of antennal segments (Table 3) when compared to the description of the neotype (Guadeloupe) and collection reference specimens.

**Table 3.** Body length, forewing length, and width and length/width ratio of antennal segments in µm of single females of *Selenothips rubrocinctus*.

Origin *	Guadeloupe	Italy	Italy	Surinam	Surinam	Vietnam	Indonesia
	Neotype	2019(1)	2019(2)	1960	2011	2018	1936
Body length	1300	1515	1285	1457	1215	1375	1415
Forewing							
-length	755	837	800	728	745	746	714
-medial width	49	57	56	55	55	49	51
Antenna (l/w)							
Ι	24/28-0.9	28/31-0.9	24/25-1.0	21/32-0.7	21/24-0.8	22/25-0.9	22/28-0.8
II	48/36-1.3	53/36-1.5	51/33-1.5	45/42-1.1	46/34-1.4	45/36-1.3	45/33-1.4
III	62/26-2.4	65/27-2.4	58/30-1.9	59/29-2.0	55/26-2.1	60/26-2.3	58/27-2.1
IV	73/24-3.0	66/26-2.5	62/26-2.4	65/26-2.5	68/25-2.7	61/25-2.4	63/23-2.7
V	42/24-1.8	41/25-1.6	37/26-1.4	40/25-1.6	42/23-1.8	38/25-1.5	38/23-1.7
VI	29/22-1.3	32/20-1.6	27/22-1.2	28/23-1.2	26/20-1.3	29/26-1.1	29/19-1.5
VII	17/13-1.3	18/11-1.6	14/13-1.1	15/13-1.2	13/13-1.0	13/10-1.3	11/>8-<1.4
VIII	34/6-5.7	31/6-5.24	26/6-4.3	29/6-4.8	24/6-4.0	26/6-4.3	26/>3-<8.7
Total length	329	334	299	302	295	294	292

\* Guadaloupe, French West Indies, Wilson (1975), others: collection Netherlands Food and Consumer Product Safety Authority. Italy (1), 5683121, Crespatica, 24-vi-2019, *Acer campestre*, leg. A. Wojnar, det. G. Vierbergen. Italy (2), 5683090, Bagnolo Cremasco, 24-vi-2019, *Carpinus*, leg. A. Wojnar, det. G. Vierbergen. Surinam, Paramaribo, x-1960, *Theobroma cacao*, leg. P. van Doesburg, det. W. P. Mantel. Netherlands, 4973723, Schiphol Airport (import Surinam), 1-iv-2011, *Codiaeum* (cuttings), leg. L. Vriens, det. G. Vierbergen. Vietnam, Hài Phòng, 8-xi-2018, *Cleistocalyx operculatus*, leg. Vu Hoa, det. G. Vierbergen. Indonesia, Java, Pasar Minggu, i-1936, det. F. André, confirmed H. Priesner. Grey colour to highlight the two italian specimens.

#### 3.2. Molecular Analysis

The PCR of mt-COI produced fragments of  $\pm$  650 bp, and, after trimming, the final alignment consisted of 597 bp. The nucleotide composition of these sequences was T(U) = 29.7%, A = 37.7%, C = 14.3%, and G = 18.3%. The average A + T content was high (67.4%), which is in agreement with values usually recorded for insects [37]. The sequences showed an identity in the range of 97.8–95.8% with S. rubrocinctus sequences available from BOLD Systems and GenBank (percentage similarity and percentage identity respectively). COI sequences obtained from adult and larval specimens collected from different host plants (L. styraciflua, C. betulus, K. paniculata, A. campestre, P. persica) in different neighbouring municipalities (Bagnolo Cremasco, Cremosano, Crespiatica, Pandino, Palazzo Pignano) showed the presence of one haplotype. However, a limited number of specimens was sequenced in those locations (Table 4). In addition to haplotype 1, a second haplotype was found in the municipality of Trescore Cremasco, where the highest number of specimens was sampled and sequenced. An identity of 99.5% with the only Parabaliothrips coluckus Kudo, 1977 accession (HM246183.1) available from GenBank was found for haplotype 1. However, this accession is not associated with any publication and no voucher specimens could be retrieved, therefore it was considered as not reliable.

**Table 4.** Distribution and abundance of *S. rubrocinctus* haplotypes in relation to host-plant and location. GenBank accession numbers related to the COI sequences of the analysed samples are indicated.

Haplotype	Number of Samples Sequenced	Host Plant	Location
	1	Carpinus betulus	Bagnolo Cremasco (1)
	1	Carpinus betulus	Bagnolo Cremasco (2)
Haplotype 1	1	Koelreuteria paniculata	Cremosano
	1	Acer campestre	Crespiatica (1)
	1	Liquidambar styraciflua	Crespiatica (2)

Haplotype	Number of Samples Sequenced	Host Plant	Location	
	1	Liquidambar styraciflua	Pandino	
	1	Parrotia persica	Palazzo Pignano (Scannabue)	
	12	Compinens hoteles	T C	
Haplotype 2	6	- Curpinus betulus	Trescore Cremasco	

Table 4. Cont.

The Neighbour-Joining tree shows that the COI sequences of the two Italian haplotypes cluster with the reference sequences published in GenBank (Figure 1).



**Figure 1.** Bootstrap consensus tree generated using the Neighbour-Joining method and general time reversible (GTR) G + I (gamma + invariant) model showing the genetic differences and relationship among *S. rubrocinctus* haplotypes obtained by mt-COI sequences. *S. rubrocinctus* sequences [38,39] available in NCBI are included and *Caliothrips indicus* was used as an outgroup. Species names and GenBank accession numbers are shown in the figure.

#### 4. Discussion and Conclusions

Thrips are considered amongst the most successful invasive insect pests, due to their small size and cryptic habits, together with a polyphagous and opportunistic behaviour [40].

There are several examples of thrips species that managed to cross biogeographical borders, most likely with unaware human assistance, and establish in previously uncolonised regions. It is the case of *Microcephalothrips abdominalis* (D. L. Crawford, 1910), a mainly tropical and subtropical species, which have been present in Italy since the end of the last century [41]. This thrips has also been found in Slovenia [42], Croatia and Hungary [43], Canary Islands [44], France [45] and Bulgaria [46]. In 2006, a permanent population of *Thrips hawaiiensis* (Morgan, 1913) was discovered outdoors in France. This represents the first report in Europe of this thrips, which is widely distributed and common in tropical Asia and the Pacific region [47]. A few years later, permanent populations of this species were discovered outdoors in Spain [48] and Italy [49]. The global increase in temperatures on the planet, together with an intensification in the trade of goods and commodities from tropical regions, is likely to facilitate this phenomenon [50,51].

In summer 2015, the pantropical, polyphagus *Selenothrips rubrocinctus* was detected on *Liquidambar styraciflua* (Figure A4 in Appendix A) and *Koelreuteria paniculata* in urban areas of Palazzo Pignano, in northern Italy. The infestation has been confirmed every year since 2015 in annual surveys and affects an urban area involving several neighbouring municipalities in the provinces of Cremona and Lodi. This shows that the insect is firmly established in the area and is able to successfully survive the winters of northern Italy, even if it has not been possible yet to determine overwintering stage and mode.

The morphological identification is solid, based on two scientific publications reporting the description of the species [27,28], which is the only one belonging to the genus Selenothrips [1]. In comparing sizes of antennal segments and forewings of a large and a small Italian specimen with collection material originating from several origins (Table 3) we could not detect promising differences. Variation in body size in S. rubrocinctus is large and numbers of specimens available from different origins for study are low. However, females of *S. rubrocinctus* from Italy can be relatively large in size and they can have relatively long fore wings (Table 3). In 1949 in Florida the same was found from females collected on oak trees [27]. The length/width ratio of antennal segments VI and VII of the large Italian specimen is rather high. Both in Italy and in Florida, where it occurs also in the northern part of the state [3], the tropical S. rubrocinctus is in its northern distribution area, and in these regions thrips may show some adaptations in their morphology. It could be hypothesised that without clear differential characters, the size of Italian females may be an adaptation to longer winter periods and/or an adaptation to live on leaves of deciduous trees. It is also possible that these size adaptations are influenced by host plant species (deciduous trees) and/or environmental factors [52]. Altogether, our data on morphology are inadequate to give this larger form a taxonomic status.

In populations of *S. rubrocinctus* males are sometimes absent. Sexual and asexual (thelytoky) reproduction has been observed, but reports suggesting thelytoky are clearly more numerous, because the number of males observed is usually very low or they are not found at all. From the Italian samples, however, we observe a high male/female ratio (0.26). At present it is not clear what consequences sexual reproduction has for fecundity of populations of leaf feeding thrips. Experiments are time consuming and their results are not always applicable under field conditions. Moreover, even fecundity experiments in the laboratory [53] and the greenhouse [54] can give opposite conclusions on the significance of higher numbers of males for populations. Krueger et al. [53] found a reduction of oviposition rate and longevity for sexually reproducing *Echinothrips americanus* Morgan, 1913 while Xiao-Wei and al. [54] found the opposite for the same thrips species. More observations on the high male/female ratio of *S. rubrocinctus* in the field may possibly lead us to understand the significance of sexual reproduction for its local survival.

The molecular analysis confirms the morphological data. The phylogenetic analysis shows genetic differences within the Italian population in relation to the COI marker. Two different COI haplotypes were detected for adult and larval specimens collected from different host plants in different municipalities. Marullo et al. [55], recently demonstrated that DNA barcoding is a reliable tool for identifying genetic differences within the same thrips population. Our results revealed some genetic variation due to the presence of two haplotypes collected in the same location (Trescore Cremasco, where more specimens were collected and barcoded) and on the same host plant (*C. betulus*). This finding supports the hypothesis that at least two introduction events have occurred in the area over the years or that the founder population was characterised by some genetic variability since its introduction. It seems likely that those first specimens were introduced into Italy on indoor plants imported from other countries by nurseries, plant shops or private buyers. *Codiaeum* plants are among the most frequently imported host plants on which this species has been

intercepted by European plant health authorities in the last decades [19–24]. The Italian haplotypes differ for 2.2–4.2% from the *S. rubrocinctus* sequences available in online genetic databases. The genetic variability within this species has never been studied, resulting in a lack of knowledge in this respect. Available barcodes are very few and information on the collection site are not always linked to the barcoded specimens in GenBank and BOLD Systems. From this data, it can be assumed that *S. rubrocinctus* displays a considerable genetic variability among populations throughout its distribution range. However, the results of a broader barcoding study of a significant number of *S. rubrocinctus* populations, sampled from summer green trees and deciduous trees throughout its distribution areas, should be conducted to confirm this genetic variability.

Few studies concerning Thysanoptera fauna associated with an urban environment have been conducted. Mirab-balou et al. [14] documents the biodiversity of thrips species in the urban green areas of Hangzhou, China, and reports high levels of damage caused by *S. rubrocinctus* on different plant species in urban parks, up until the early drop of the leaves. In Italy, the polyphagous behavior of this species is confirmed by the wide range of plant hosts attacked in the infested area, belonging to different botanical families (Altingiaceae, Betulaceae, Sapindaceae, Hamamelidaceae, Fagaceae, Rosaceae). The presence on Liquidambar styraciflua, Carpinus betulus, Koelreuteria paniculata, Acer campestre, Parrotia persica was confirmed by sampling and identification of specimens. The presence on other host plants was well documented through photographic material: Sorbus aucuparia L., Quercus robur L., Castanea sativa Mill., and Acer platanoides L. (Figure A5B–F in Appendix A). The simultaneous presence of different stages of the insect on the same leaves/plant proves that the thrips is able to complete its life cycle on all the above-mentioned host plant species. Attacked leaf areas show clear depigmentation and take on a silvery colour to the upper leaf surface, with distortion of the lamina and, in the most severe cases, premature leaf drop (phylloptosis). In addition, the pest covers the underside with droplets of excrement during normal trophic activity. It has been noted that plants in a state of vegetative stress due to drought or excessive canopy reduction through cutting large branches or apices seem more susceptible to infestation, as already observed by other authors [56]. A strategy for the management and the containment of this pest on tall trees in urban contexts seems difficult to achieve. It is possible that the thrips will naturally spread on a larger scale, with the risk of affecting not only forest species used in roadside plantings, public parks, and private gardens, but also plants of agricultural interest, such as Vitis vinifera, whose attractiveness to *S. rubrocinctus* has been documented in Brazil [10–12].

This is the first time that this tropical species has been found at such a high latitude in the temperate climate zone. It is likely that climate change has contributed to the establishment of outdoor *S. rubrocinctus* populations in northern Italy. Warming climate and the resulting changes in the composition of native biological communities (biome shifts) can facilitate invasions and the establishment of allochthonous species by increasing resource availability and habitat suitability [50,51,57].

Further studies are needed to monitor the spread and shed light on the biological cycle of *S. rubrocinctus* in northern Italy, in particular focusing on overwintering mode, number of generations per year, and host plant range. Although infestation alone is unlikely to lead to tree death, it is clear that severe infestations on trees already under vegetative stress can affect not only their appearance in the urban landscape but also compromise their viability through a significant reduction in their photosynthetic capacity.

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# Appendix A



**Figure A1.** Male (**A**) and female (**B**) of *S. rubrocinctus* in microscopic slide; specimen collected from *Carpinus betulus* at Bagnolo Cremasco, 2019. Photos G. Vierbergen.



**Figure A2.** (**A**) tergites VII-X of male *S. rubrocinctus;* specimen collected from *Carpinus betulus* at Bagnolo Cremasco, 2019; (**B**) thoracial nota of female *S. rubrocinctus,* specimen collected from *Liquidambar styraciflua* at Pandino, 2019. Photos G. Vierbergen.



**Figure A3.** (**A**) larva I of *S. rubrocinctus* in microscopic slide, specimen collected from *Liquidambar styraciflua* at Crespiatica, 2019; (**B**) larva II of *S. rubrocinctus* in microscopic slide, specimen collected from *Koelreuteria paniculata* at Cremosano, 2019. Photos G. Vierbergen.



**Figure A4.** (**A**,**B**) Symptoms of *S. rubrocinctus* infestation on the lower and upper side of *Liquidambar styraciflua* leaves (Pandino, 2019); (**C**,**D**) effects of intense *S. rubrocinctus* infestation on *Liquidambar styraciflua* (Palazzo Pignano, 2015). Photos A. Wojnar.



**Figure A5.** Symptoms of *S. rubrocinctus* infestation on the lower and upper side of leaves on different host plants: (A) *Carpinus betulus* (Bagnolo Cremasco, 2015); (B) *Acer platanoides* (Palazzo Pignano, 2019); (C) *Sorbus aucuparia* (Palazzo Pignano, 2018); (D) *Quercus robur* (Palazzo Pignano, 2015); (E,F) *Castanea sativa* (Palazzo Pignano, 2015). Photos A. Wojnar.

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