Disentangling arthropod and plant resources consumed by *Orius* spp. in peach and alfalfa crops by metagenomic analysis

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Table S1. Arthropod (ZBJ-ArtF1c/ZBJ-ArtR2c and mlCOIintF/HC02198) and plant (ITS-S2F/ITS4R and cA49325/trnL110R) primer pairs used in this study. Also indicated the sequence of each forward (F) and reverse (R) primer and the length of the amplified fragment.

	Sequence 5´- 3´(F)	Sequence 5´- 3´(R)	Reference	Fragment (bp)
ZBJ-ArtF1c/ZBJ-ArtR2c	AGATATTGGAACWTTATATTTTATTTTTGG	WACTAATCAATTWCCAAATCCTCC	Zeale et al. 2011	157
mlCOIintF/HC02198	GGWACWGGWTGAACWGTWTAYCCYCC	TAAACTTCAGGGTGACCAAAAAATCA	Leray et al. 2013/Folmer et al. 1994	313
ITS-S2F/ITS4R	ATGCGATACTTGGTGTGAAT	TCCTCCGCTTATTGATATGC	Chen et al. 2010/White et al. 1990	350
cA49325/trnL110R	CGAAATCGGTAGACGCTACG	GATTTGGCTCAGGATTGCCC	Taberlet et al. 2007/Borsch et al. 2003	80

Table S2. Accession numbers of the sequences from the databases used as reference to obtain the percentages of similarity.

Accession number	species	Databases used
MG007856.1	*Orius laevigatus*	GenBank
MG007857.1		
KM021482.1	*Orius niger*	
KM021745.1		
KM021890.1		
KM022197.1		
KM022760.1		
KM022882.1		
KM022999.1		
SONE138-11	*Orius majusculus*	Bold
SONE141-11		

Table S3. Percentages of relative read abundance (RRA%) of the reads amplified from resources consumed by each arthropod and plant primer pairs in each library (L) in the trials included in the study: **(1)** Arthropod taxa detected in the analyzed *Orius*; **(2)** Plant taxa detected in the anlyzed *Orius* Those percentages eliminated from the analysis for exceeding the 1% threshold are shown in bold and grey. Art1= ZBJ-ArtF1c/ZBJ-ArtR2c; Art2= mlCOIintF/HC02198; Pl1= ITS-S2F/ITS4R; Pl2= cA49325/trnL110R; NA= Not amplified.

(1)

Primers used	**L1**		**L3**		**L5**		**L7**		**L9**		**L11**	
Taxa detected	**Art1**	**Art2**	**Art1**	**Art2**	**Art1**	**Art2**	**Art1**	**Art2**	**Art1**	**Art2**	**Art1**	**Art2**
Orius	-	-	-	NA	-	-	-	-	-	NA	-	-
Orius laevigatus	-	-	100	NA	8,88	-	-	-	-	NA	87,23	-
Orius majusculus	-	-	-	NA	-	-	-	-	-	NA	-	-
Orius niger	-	-	-	NA	-	-	-	-	-	NA	-	-
Aeolothrips intermedius	-	-	-	NA	-	-	73,78	16,72	-	NA	**0,21**	-
Frankliniella occidentalis	-	100	-	NA	-	100	-	74,62	-	NA	-	73,11
Aphididae	100	-	-	NA	-	-	-	-	-	NA	-	-
Therioaphis trifolii	-	-	-	NA	-	-	-	-	-	NA	**0,29**	10,27
Hypera	-	-	-	NA	5,33		2,32	-	-	NA	-	-
Liriomyza	-	-	-	NA	3,55		21,58	8,66	-	NA	-	-
Aedes caspius	-	-	-	NA	-	-	-	-	-	NA	**0,38**	8,07
Ceratopogonidae	-	-	-	NA	-	-	-	-	-	NA	**0,21**	4,89
Tanytarsus volgensis	-	-	-	NA	68,64	-	-	-	-	NA	-	-
Orthocladiinae	-	-	-	NA	13,61	-	2,32	-	-	NA	-	-
Cicadellidae	-	-	-	NA	-	-	-	-	100	NA	11,68	-

(2)

Primers used	**L2**		**L4**		**L6**		**L8**		**L10**		**L12**	
Taxa detected	**Pl1**	**Pl2**	**Pl1**	**Pl2**	**Pl1**	**Pl2**	**Pl1**	**Pl2**	**Pl1**	**Pl2**	**Pl1**	**Pl2**
Streptophyta	-	-	NA	-	-	-	NA	-	-	2,17	100	31,69
Asparagales	-	-	NA	-	-	7,07	NA	-	-	-	-	-
Caryophyllales	-	-	NA	-	-	-	NA	-	-	-	-	8,69
Asteraceae	-	-	NA	12,95	-	2,18	NA	-	-	42,04	-	-
Poaceae	-	-	NA	-	-	1,25	NA	-	-	-	-	-
Solanaceae	-	2,67	NA	-	-	-	NA	-	-	-	-	-
Poa annua	-	-	NA	-	-	-	NA	-	-	1,78	-	-
Pinus	-	9,45	NA	15,83	-	2,18	NA	-	-	-	-	1,36
Fabaceae	-	40,86	NA	41,73	-	87,32	NA	100	-	42,93	-	58,26
Rosaceae	-	47,02	NA	29,50	-	-	NA	-	-	11,08	-	-
Medicago sativa	-	-	NA	-	100	-	NA	-	59,65	-	-	-
Prunus persica	100	-	NA	-	-	-	NA	-	40,35	-	-	-

Table S4. Taxa obtained from each library and with each primer pair. NT= not tested. NAmp = Not amplified.

species/sample	Crop	Date	# of individuals/ Sample size	Library #	Arthropod primer pairs		Plant primer pairs	
					ZBJ-ArtF1c/ZBJ-ArtR2c	mlCOIintF/HC02198	ITS-S2F/ITS4R	CA49325/trnL110R
Orius laevigatus	Peach	June-2016	7	L1	*Orius laevigatus* Aphididae	*Orius* *Orius laevigatus* *Frankliniella occidentalis*	NT	NT
				L2	NT	NT	*Prunus persica*	Solanaceae *Pinus* Rosaceae Fabaceae
Orius majusculus			21	L3	*Orius laevigatus* *Orius majusculus*	*Orius* *Orius majusculus*	NT	NT
				L4	NT	NT	NAmp	Asteraceae *Pinus* Fabaceae Rosaceae
Orius niger	Alfalfa	June-2016	9	L5	*Hypera* *Tanytarsus volgensis* Orthocladiinae *Liriomyza* *Orius laevigatus* *Orius niger*	*Orius niger* *Frankliniella occidentalis*	NT	NT
				L6	NT	NT	Streptophyta	Streptophyta Caryophyllales Fabaceae *Pinus*
		June-2016	25	L7	*Hypera* Orthocladiinae *Liriomyza* *Orius niger* *Aeolothrips intermedius*	*Liriomyza* *Orius niger* *Frankliniella occidentalis* *Aeolothrips intermedius*	NT	NT

				L8	NT	NT	*Medicago sativa*	Asparagales Asteraceae Poaceae *Pinus* Fabaceae
		July-2017	13	L9	*Orius* *Orius niger* Cicadellidae	*Orius* *Orius niger*	NT	NT
				L10	NT	NT	NAmp	Fabaceae
		September-2017	22	L11	*Orius* *Orius laevigatus* *Orius niger* Cicadellidae	*Aedes caspius* Ceratopogonidae *Orius* *Orius niger* *Therioaphis trifolii* Cicadellidae *Frankliniella occidentalis*	NT	NT
				L12	NT	NT	*Medicago sativa* *Prunus persica*	Streptophyta Asteraceae Fabaceae Rosaceae *Poa annua*
Prunus persica	Peach		1 cm^2	L13	NT	NT	*Prunus persica*	Rosaceae
Medicago sativa	Alfalfa		1 cm^2	L14	NT	NT	*Medicago sativa*	Fabaceae

Table S5. Percentages of similarity means by pairs from the region amplified by each arthropod primer pair used calculated from the sequences obtained in the databases of three amplified *Orius* species.

Species comparison	**ZBJ-ArtF1c/ZBJ-ArtR2c (%)**	**mlCOIintF/HC02198 (%)**
O. majusculus - O. laevigatus	92,04	87,29
O. majusculus - O. niger	92,42	90,85
O. laevigatus - O. niger	94,34	91,53

Table S6. Mean of the calculated percentages of similarity between the obtained number of OTUs for the predator (*O. majusculus* or *O. niger*) and for the prey (other *Orius* species) using the ZBJ-ArtF1c/ZBJ-ArtR2c pair of primers.

Predator species	Prey species	Library	# Predator OTUs	# Prey OTUs	Mean % similarity between OTUs
O. majusculus	*O. laevigatus*	L3	5	5	91,90
O. niger	*O. laevigatus*	L5	6	1	92,73
O. niger	*O. laevigatus*	L11	2	1	93,94

Table S7. Number of reads and OTUs obtained per library regarding the amplified *Orius* species, and the taxon finally assigned with each pair of arthropod primer used. The species with the highest number of reads and OTUs within a library and pair of primer used is indicated in grey.

Predator	Library	Primer pair	*Orius* species		
			# Reads	**# OTUs**	**Taxon assigned**
Orius laevigatus	L1	ZBJ-ArtF1c/ZBJ-ArtR2c	56868	23	***O. laevigatus***
		mlCOIintF/HC02198	2015	3	***O. laevigatus***
Orius majusculus	L3	ZBJ-ArtF1c/ZBJ-ArtR2c	24906	5	***O. majusculus***
			6668	5	*O. laevigatus*
		mlCOIintF/HC02198	1635	2	***O. majusculus***
Orius niger	L5	ZBJ-ArtF1c/ZBJ-ArtR2c	109935	6	***O. niger***
			15	1	*O. laevigatus*
		mlCOIintF/HC02198	10679	2	***O. niger***
	L7	ZBJ-ArtF1c/ZBJ-ArtR2c	79012	3	***O. niger***
		mlCOIintF/HC02198	21255	2	***O. niger***
	L9	ZBJ-ArtF1c/ZBJ-ArtR2c	25677	1	***O. niger***
		mlCOIintF/HC02198	19202	1	***O. niger***
	L11	ZBJ-ArtF1c/ZBJ-ArtR2c	38716	2	***O. niger***
			3668	1	*O. laevigatus*
		mlCOIintF/HC02198	23446	38	***O. niger***
		Total	423697	95	

Table S8. Number of template-mismatches of each arthropod pair of primers with each amplified taxon. NS = no sequence present in the databases.

	mlCOIintT/HC02198		**ZBJ-ArtF1c/ ZBJ-ArtR2c**	
Taxon	**Forward (mlCOIintT)**	**Reverse (HC02198)**	**Forward (ZBJ-ArtF1c)**	**Reverse (ZBJ-ArtR2c)**
Hypera	1	NS	0	2
Liriomyza	1	3	1	1
Tanytarsus volgensis	1	NS	3	8
Orthocladiinae	0	NS	0	6
Ceratopogonidae	1	NS	3	4
Aedes caspius	1	1	1	5
Orius	2	4	3	3
Orius laevigatus	0	NS	1	0
Orius majusculus	1	NS	1	0
Orius niger	0	NS	0	2
Aphididae	1	NS	3	2
Therioaphis trifolii	2	NS	3	8
Cicadellidae	1	NS	4	2
Aeolothrips intermedius	0	NS	3	6
Frankliniella occidentalis	1	NS	4	5

Figure S1. Percentage of predator and prey taxa amplified by each arthropod primer pairs from each library: **(A)** Taxa amplified by ZBJ-ArtF1c/ZBJ-ArtR2c primer pairs; **(B)** Taxa amplified by mlCOIintF/HC02198 primer pairs. Predator (green); Prey (orange).

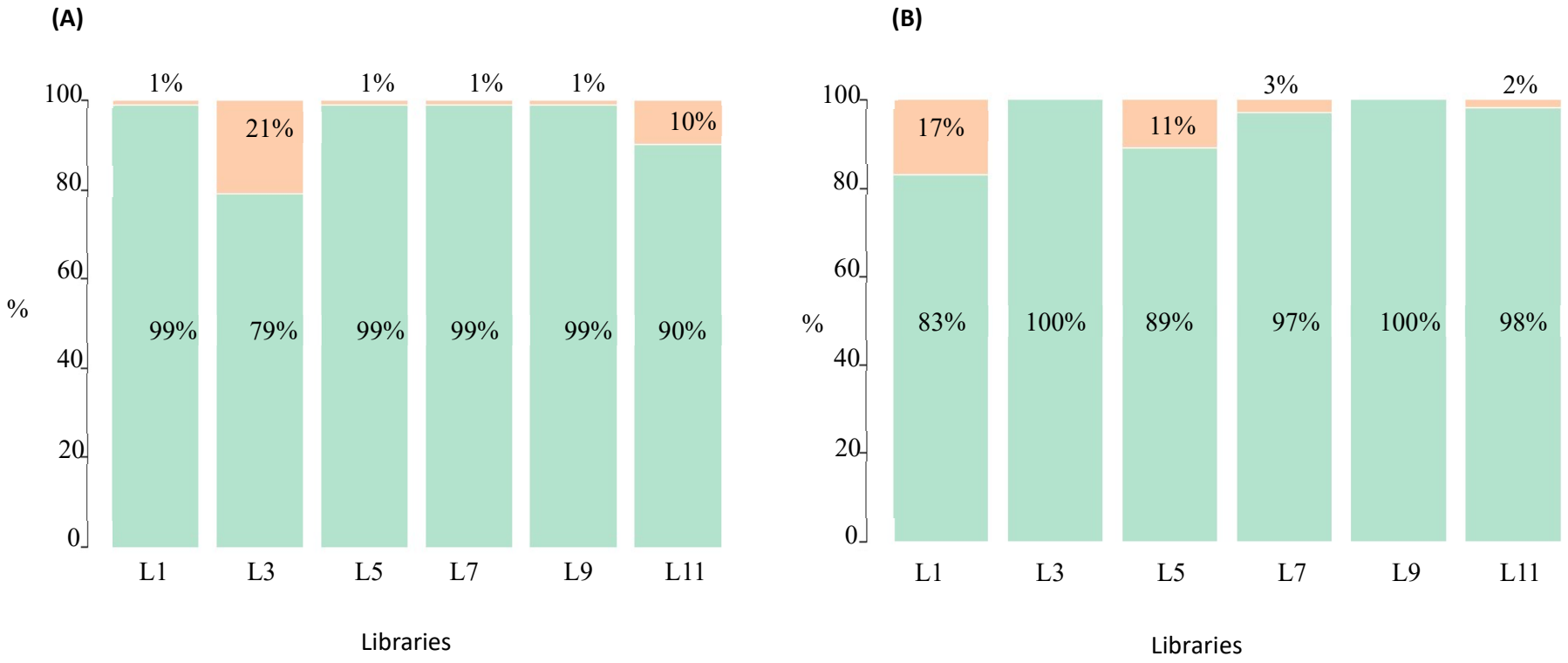
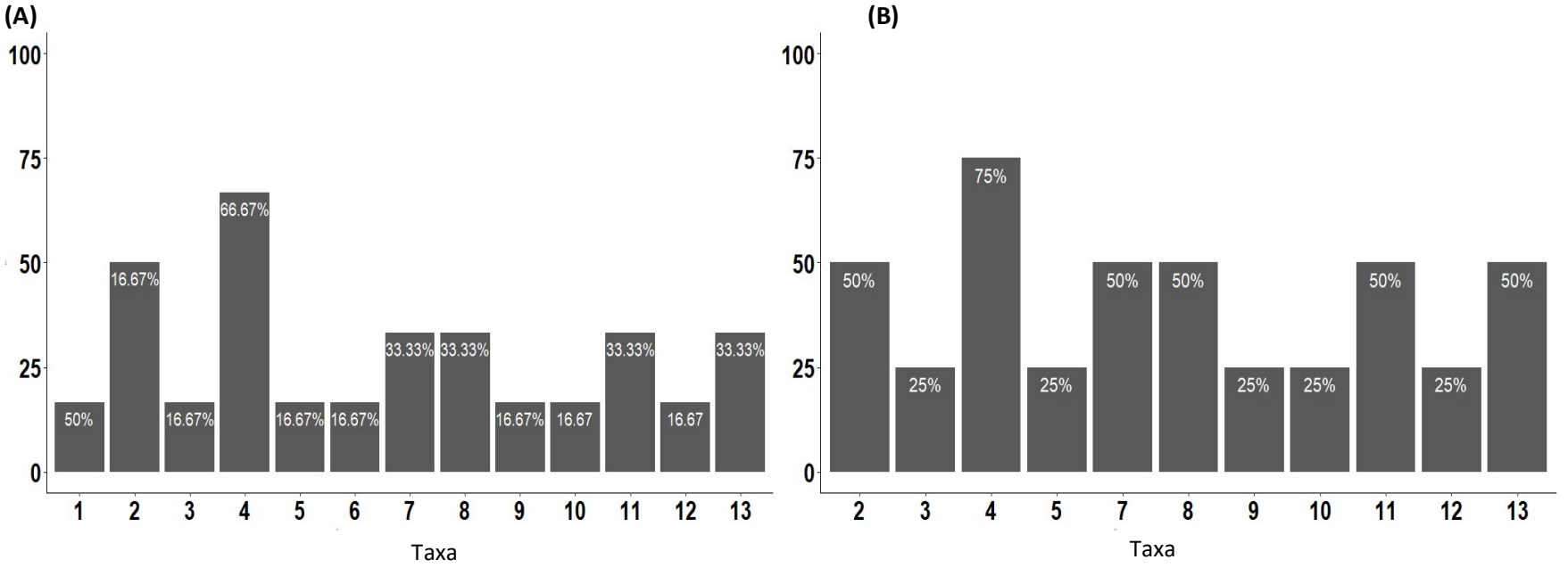


Figure S2. Percentage of frequency of occurrence (FOO%) of the **(A)** arthropod taxa resources items consumed by all analysed *Orius*; **(B)** arthropod taxa resources items consumed by *Orius niger;* **(C)** plant taxa resources items consumed by all *Orius*; **(D)** of the plant taxa resources items consumed by *Orius niger***.**



1-*Orius*

2-*Orius laevigatus*

3-*Aeolothrips intermedius*

4-Frankliniella occidentalis

5-Aphididae

6- *Therioaphis trifolii*

7-*Hypera*

8-Liriomyza

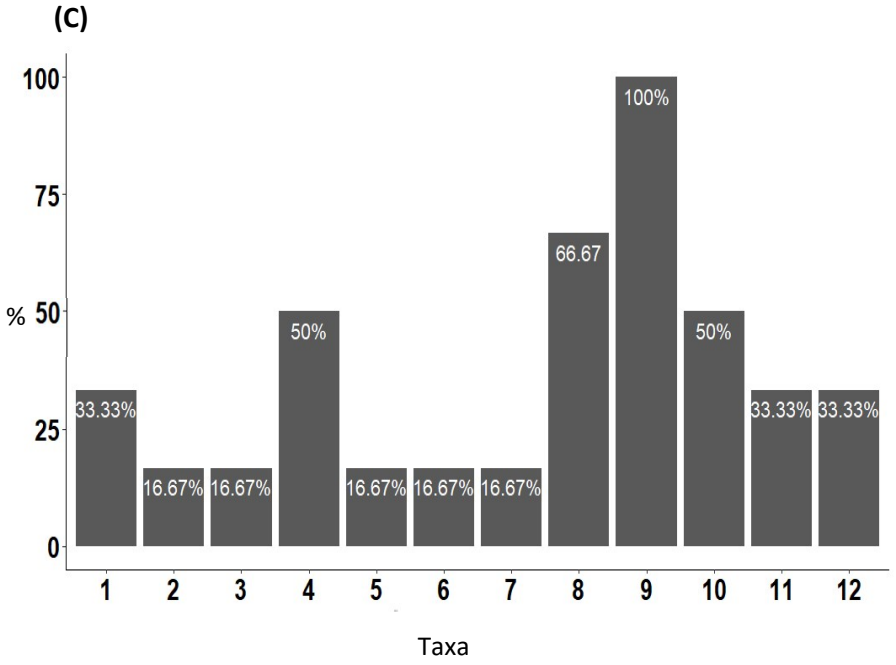
9- *Aedes caspius*

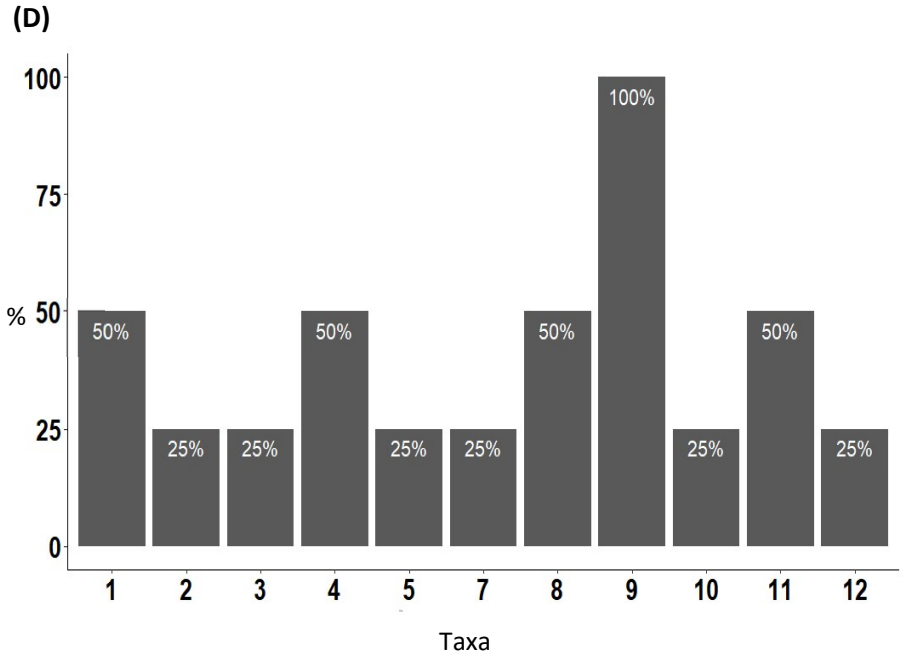
10- Tanytarsus volgensis

11- Orthocladiinae

12- Ceratopogonidae

13-Cicadellidae





1-Streptophyta	4-Asteraceae	7-*Poa annua*	10-Rosaceae
2-Asparagales	5-Poaceae	8-*Pinus*	11-*Medicago sativa*
3-Caryophyllales	6-Solanaceae	9-Fabaceae	12-*Prunus persica*