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Development of molecular tools to identify the main weevil pests of oilseed rape and their natural enemies.

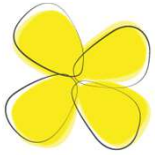
Robert C., Bothorel S., Luce S., Lauvernay A., Leflon M., Delvare G., Streito J.C., Pierre E., Cruaud P., Ollivier M., Genson G., Cruaud A., Rasplus J.Y.

Contact : c.robert@terresinovia.fr



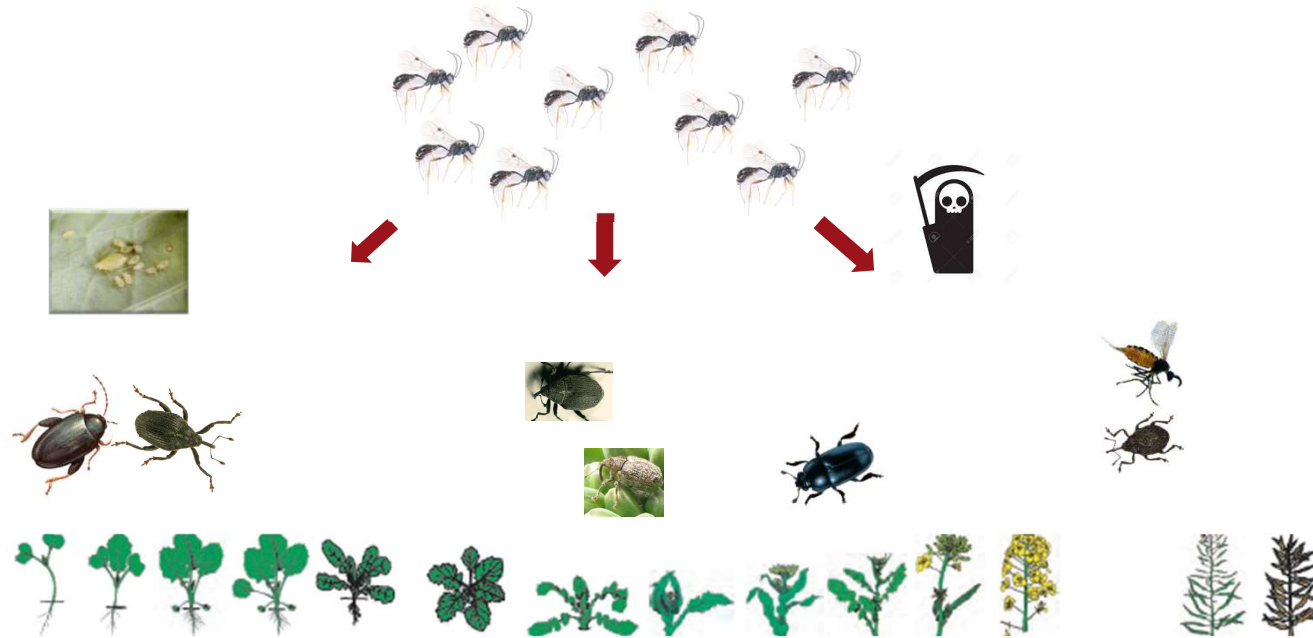
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OSR pests regulated by many parasitoids

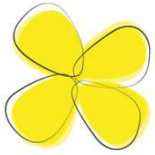
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- Natural enemies can help farmers to regulate pests populations in their fields -> **conservation biological control.**
- To promote their action, a good knowledge of their biology is essential.

➔ **Tools to identify species and quantify parasitism rates are needed**
-> **Coleotool Project**





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A massive field collection for two years to create the database

Trapping



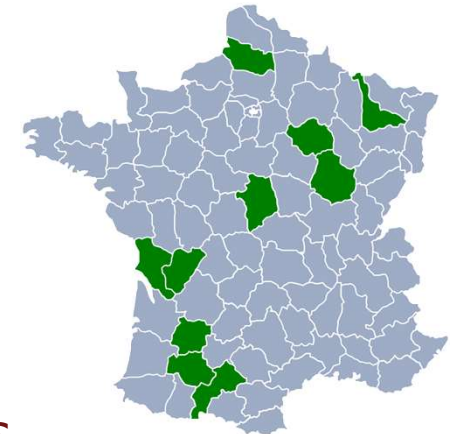
Rearing



159 collected samples in spring and in autumn 2014, in most French regions



35 larval populations of weevils were reared in 2014 and 2015 in most French regions



Sampling location





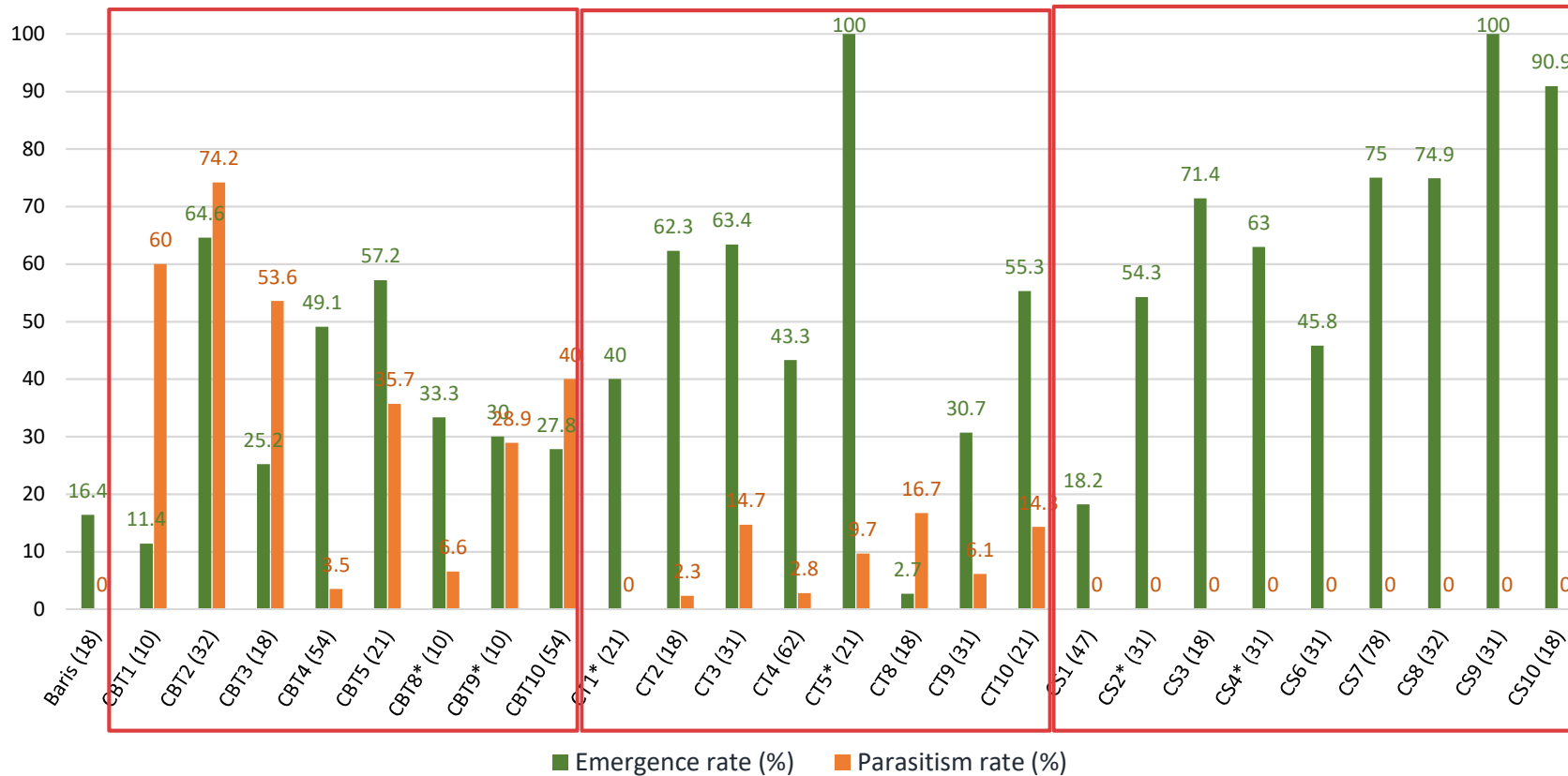
Updated references on endoparasitism rates (PR)

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C. picitarsis
PR mean = 37.8 (sd=24.8)

C. napi or pallidactylus
PR mean = 8.3 (sd=6.4)

C. obstructus
PR mean = 0 (sd=0)



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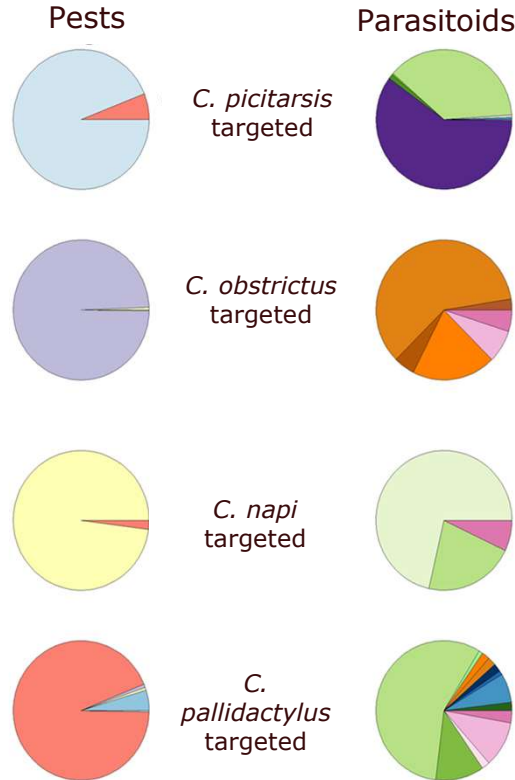


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Updated references on the parasitoids species and their territorial distribution



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Identified pests

- Aulacobaris coeruleascens*
- Ceutorhynchus napi*
- Ceutorhynchus obstrictus*
- Ceutorhynchus pallidactylus*
- Ceutorhynchus pictarsis*

Identified parasitoids

- Anaphes sp. 2*
- Aneucis melanaria*
- Diospilus capito*
- Diospilus oleraceus*
- Diospilus sp.*
- Mesopolobus morys*
- Mesopolobus sp.*
- Necremnus hippia*
- Stenomalina gracilis*
- Tersilochus fulvipes*
- Tersilochus heterocerus*
- Tersilochus obscurator*
- Tersilochus*
- Tersilochus stenocari*
- Triaspis caudata*
- Triaspis*
- Trichomalus apertus*
- Trichomalus perfectus*
- Trichomalus sp.*

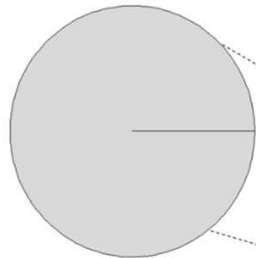


Updated references on the parasitoids species and their territorial distribution

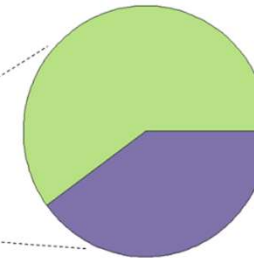
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Proportion of the different parasitoid species in samples targeting *C. picitarcis* (2014)

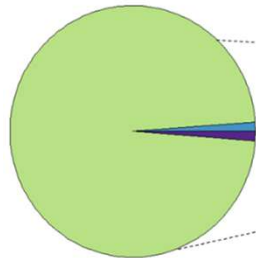
Number of parasitoids = 4



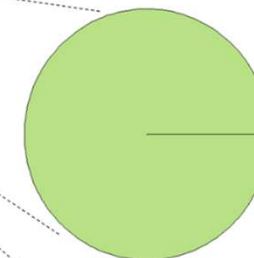
Number of parasitoids = 5
PR rate = 10%



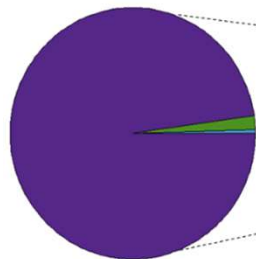
Number of parasitoids = 82
PR rate = 15%



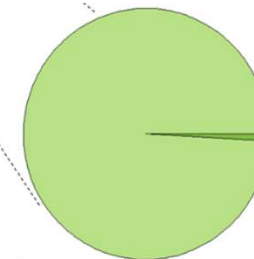
Number of parasitoids = 12
PR rate = 19%



Number of parasitoids = 394
PR rate = 20%

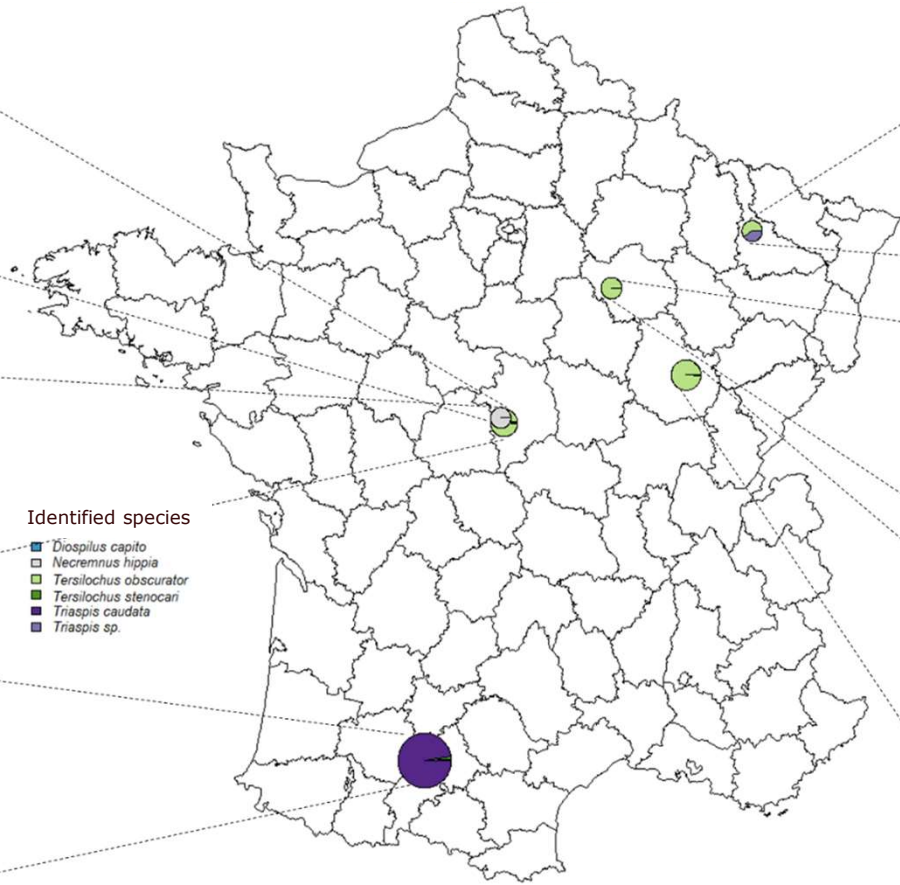


Number of parasitoids = 114
PR rate = 12%



Identified species

- *Diospilus capito*
- *Necremnus hippia*
- *Tersilochus obscurator*
- *Tersilochus stenocari*
- *Triaspis caudata*
- *Triaspis sp.*





Creation of a morphological identification key for weevils (9 sp) and their parasitoids (23 sp)

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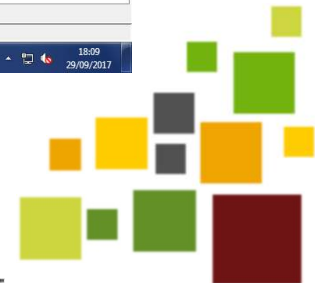


To identify parasitoids, the host must be known

<http://www1.montpellier.inra.fr/CBGP/coleotool/index.html>



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Creation of a morphological identification key for weevils and their parasitoids

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To identify parasitoids, the host must be known

The screenshot displays the COLEOTOOL web application. The browser address bar shows the URL www1.montpellier.inra.fr/CBGP/coleotool/key/cleparasitoides.html. The page title is "COLEOTOOL : Parasitoides". The interface includes a search bar, a sidebar with navigation links, a central feature selection tree, and two panels on the right: "Entites Remaining: 5" and "Entites Discarded: 19". An inset image shows a weevil with labels for *Tersilochus obscurator* and *Tersilochus microgaster*.

<http://www1.montpellier.inra.fr/CBGP/coleotool/index.html>



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Creation of a morphological identification key for weevils and their parasitoids

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To identify parasitoids, the host must be known

The screenshot displays the COLEOTOOL web interface for parasitoid identification. The browser address bar shows the URL: www1.montpellier.inra.fr/CBGP/coleotool/key/cleparasitoides.html. The interface is divided into several sections:

- Left Panel:** A sidebar with navigation options like 'Barre personnelle', 'Leica IC80 HD', 'Cérix XenApp', 'Arthemis DB@se on Arthropod Ecology...', 'Lonchaeidae Online', 'Wikis', 'Install Software', 'Intranet', 'COLEOTOOL : Accueil', 'Menu des marque-pages', and 'Autres marque-pages'.
- Top Panel:** A search bar and a list of 'Entités Remaining: 5', including *Tersilochus obscurator* (Aubert 1959) and *Tersilochus microgaster* (Szepilgib 1988).
- Central Panel:** A large image of a parasitoid, *Tersilochus obscurator* (Ichneumonidae, Tersilochinae), with a scale bar of 800 µm. The image is titled 'Tersilochus obscurator (Aubert 1959) - Lucid3'.
- Right Panel:** A list of 'Features Available: 31' and 'Features Chosen: 21'. The 'Features Available' list includes:
 - Moins de 4
 - 4 ou plus
 - Cellule costale
 - Présente mais ouverte sur l'extérieur
 - Absente
 - Ptérostigma
 - Présent
 - Absent
 - Nervure 2m-cu
 - Présente (parfois partiellement pigmentée)
 - Absente
 - 2ème cellule submarginale
 - Présente
 - Absente
 - Cellule subdiscoidale
 - Ouverte
 - Fermée (un pli de faille peut donner l'impression d'une légère)
 - Cellule submarginale 1 et discoidale 1
 - Fusionnées
 - Séparées
 - Position relative de 2m-cu par rapport à 1r-m (la division des nervures R)
 - Basale
 - Opposée ou apicale
 - Aile postérieure
 - Patte
- Bottom Panel:** A list of 'Features Chosen: 21' including:
 - Corps à reflets métalliques
 - Oui
 - Non
 - Taille du corps (du sommet de la tête à l'extrémité de l'abdomen)
 - Supérieure à 1 mm
 - Inférieure à 1 mm
 - Antennes
 - Forme du scape
 - Court et globuleux
 - Nombre d'articles antennaires
 - Supérieur à 15
 - Nombre d'anelli
 - 0 ou 1
 - 2
 - 3
 - Antennes coupées entre scape et pédicelle
 - Non
 - Insertions antennaires
 - Eboitement séparés
 - Tête
 - Front et vertex
 - Pourvus de sutures (formant un H)
 - Dépourvus de sutures
 - Mésosoma
 - Prepectus (séparant le pronotum de la tegula)

<http://www1.montpellier.inra.fr/CBGP/coleotool/index.html>



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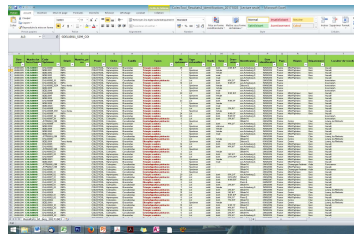
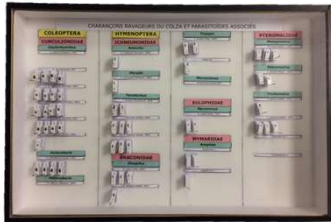


Creation of a database containing 149 validated sequences of weevils and 177 of their parasitoids

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Identification by entomologists

Specimen collection



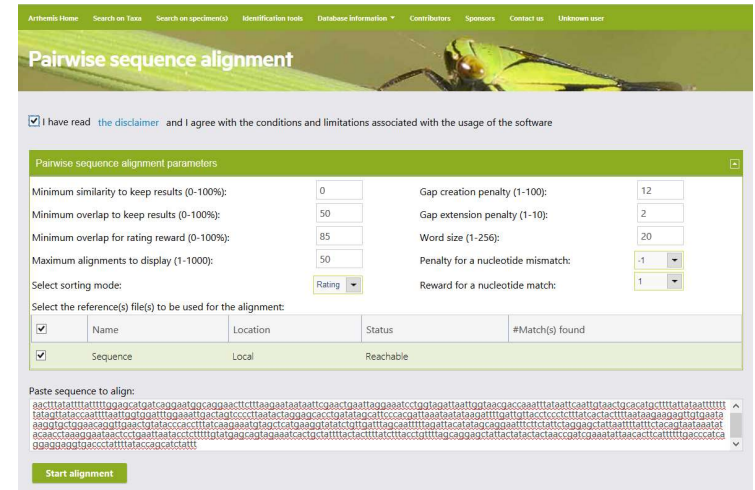
Sequencing and validation

CO1 gene



Latin names	Number of validated sequences (number of identified specimens)
WEEVILS	
<i>Ceutorhynchus pallidactylus</i>	35 (996)
<i>Ceutorhynchus napi</i>	32 (511)
<i>Ceutorhynchus pictarisis</i>	19 (633)
<i>Ceutorhynchus obstrictus</i>	39 (1199)
<i>Ceutorhynchus assimilis</i>	7 (17)
<i>Ceutorhynchus typhae</i>	5
<i>Aulacobaris coerulescens</i>	12 (381)
PARASITOIDS	
Braconidae	
<i>Diospilus sp.</i>	0 (2)
<i>Diospilus capito</i>	9 (14)
<i>Diospilus oleraceus</i>	1 (1)
<i>Triaspis sp.</i>	7 (10)
<i>Triaspis caudata</i>	19 (435)
Eulophidae	
<i>Necremnus hippia</i>	3 (4)
Ichneumonidae	
<i>Aneucis melanaria</i>	2 (3)
<i>Phradis spp.</i>	2 (4)
<i>Phradis mononellus</i>	1 (2)
<i>Tersilochus sp.</i>	0 (19)
<i>Tersilochus fulvipes</i>	6 (10)
<i>Tersilochus heteroceris</i>	0 (2)
<i>Tersilochus obscurator</i>	76 (311)
<i>Tersilochus stenocari</i>	7 (7)
Mymaridae	
<i>Anaphes sp. 1</i>	3 (3)
<i>Anaphes sp. 2</i>	1 (1)
Pteromalidae	
<i>Mesopolobus morys</i>	16 (26)
<i>Mesopolobus sp.</i>	1 (2)
<i>Stenomalina gracilis</i>	5 (10)
<i>Trichomalus sp.</i>	2 (6)
<i>Trichomalus apertus</i>	2 (3)
<i>Trichomalus lucidus</i>	5 (5)
<i>Trichomalus perfectus</i>	9 (16)

Database creation (available online -> Artemis database)



<http://arthemisdb.supagro.inra.fr/>



Creation of a database containing 149 validated sequences of weevils and their parasitoids

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Arthemis Home Search on Taxa Search on specimen(s) Identification tools Database information Contributors Sponsors Contact us Unknown user

Pairwise sequence alignment

I have read [the disclaimer](#) and I agree with the conditions and limitations associated with the usage of the software

Pairwise sequence alignment parameters

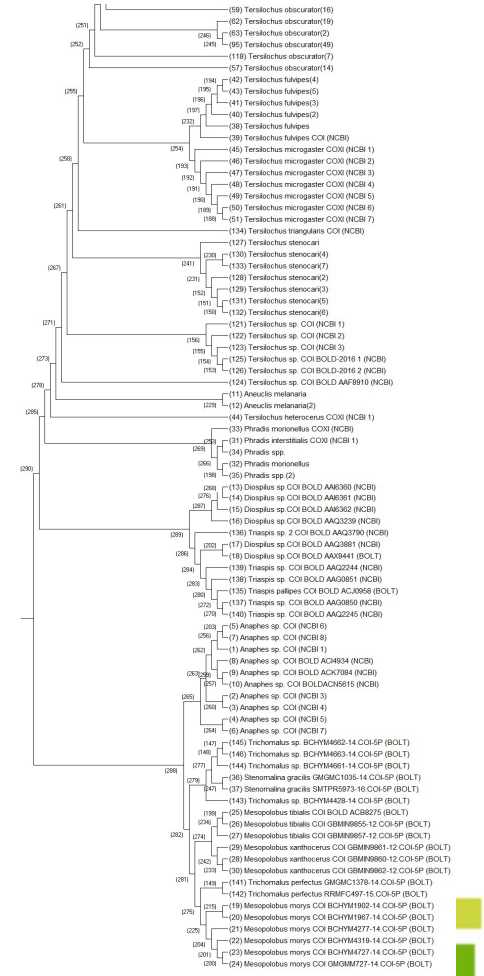
Paste sequence to align:

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aaactttatattttttggagcatgatcaggaatggcaggaactcttaagaataaataatcgaaactgaataggaaatcctggtagatataatggtaacgaccaaattataatcaattgtaactgcacatgcttttataatttttt
tatagttataccaatttaattggtagatttggaaatgactagcccccttaactagggagcactgatataagcattcccagatlaaataataaagatttggatttaccctcttatacactacttataaagaagattggaata
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acaacctaagggaataaactcctgaataaactctttttgatgagcagtgaataactcactgattttactacttttacttctggtttgacggagctactactactaaccgatcgaataatcaaccctttttgaccatca
ggagqaggtgaccctattttataccagcatctatt
```

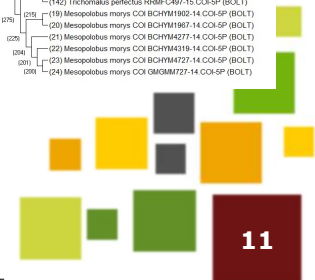
Start alignment

Draw Tree Expand Alignments Export data

Pairwise Results		Clustering results						
#	Reference description	Score	Probability	Similarity%	Fragments	Overlap%	Direction	Rating
1	JSTR02652_0103 Ceutorhynchus napi, nlink892: COI Sequence	1044.49	0	100	1	100	+/+	*****
2	EPIE00592_0102 Ceutorhynchus napi, nlink892: COI Sequence	1041.32	0	99.848	1	100	+/+	****
	EPIE00592_0104							



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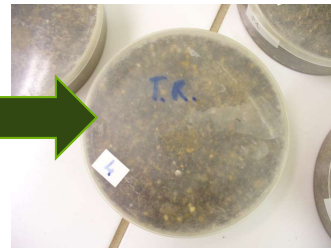
Development of molecular identification tools

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Specimen collection

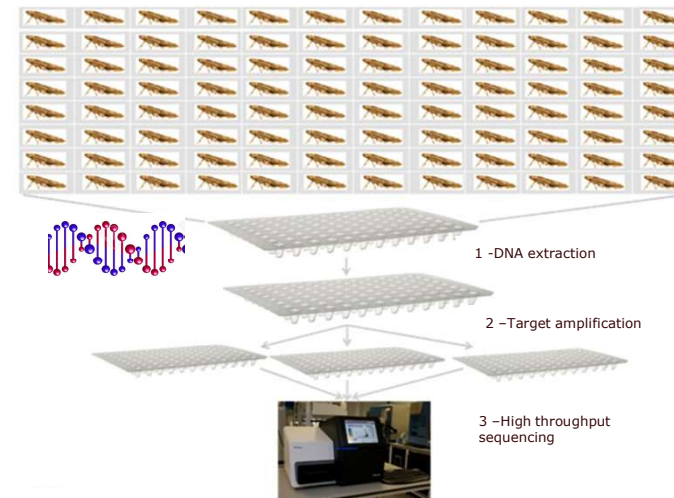



Rearing

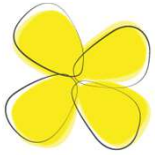
Quantification of parasitism rates by rearing parasitized weevil larvae

High throughput sequencing methods



- Allow the sequencing of several hundred insects in parallel
 - Cost reduction
- Allow to study trophic relationship





Outlooks

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- Development of simplified molecular methods to identify specimens at any stage and quantify parasitism rates on a regular basis (quick results, easy to use...)
- Development of tools for *Psylliodes chrysocephala* and their parasitoids.
- Practical application of the developed methods in the R2D2 project (poster)



Tools available online on the Coleotool website

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<http://www1.montpellier.inra.fr/CBGP/coleotool/index.html>

Sheets on species

Search on : Taxon thesaurus INRA

Add condition Match on All conditions Reset base condition(s) Switch to Advanced Search Search

Search conditions (click to expand)

Collapse titles Export data

Terislochus fulvipes	
Taxonomic information	
Complete taxon name	Terislochus (Terislochus) fulvipes (Gravenhorst, 1829)
Taxonomic rank	species
Classification	Animalia Eumetazoa, Arthropoda, Hexapoda, Insecta, Hymenoptera, Apoidea, Ichneumonidae, Ichneumonidae, Terislochinae, Terislochus (Terislochus)
Associated records	None
Taxon validity	nomen valid
Junior synonym(s)	1. Porizon fulvipes, 2. Terislochus gallicator, 3. Terislochus gallicator
Taxon iconography	

Link to:

<http://arthemisdb.supagro.inra.fr/>

Morphological identification key

Sequence database

Pairwise sequence alignment

I have read the disclaimer and I agree with the conditions and limitations associated with the usage of the software

Pairwise sequence alignment parameters

Minimum similarity to keep results (0-100%): 0
Minimum overlap to keep results (0-100%): 50
Minimum overlap for rating reward (0-100%): 85
Maximum alignments to display (1-1000): 50

Gap creation penalty (1-100): 12
Gap extension penalty (1-10): 2
Word size (1-256): 20
Penalty for a nucleotide mismatch: -1
Reward for a nucleotide match: 1

Select sorting mode: Rating

Select the reference(s) file(s) to be used for the alignment:

<input type="checkbox"/>	Name	Location	Status	#Match(s) found
<input checked="" type="checkbox"/>	Sequence	Local	Reachable	

Paste sequence to align:

Start alignment

Link to:

<http://arthemisdb.supagro.inra.fr/>

Thank you for your attention !



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