COST Action 0807
Psyllid Training School

Working group 2 “Insect vectors”

Neustadt / W., Germany 19th to 23rd of April 2010

***AlPlanta* – Institute for Plant Research**

Naturhistorisches Museum Basel

Gilat Research Center

Organisers

RLP AgroScience GmbH, AlPlanta – Institute for Plant Research
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Naturhistorisches Museum Basel
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Supported by

COST Action 0807: Integrated Management of Phytoplasma Epidemics in Different Crop systems

RLP AgroScience GmbH, AlPlanta – Institute for Plant Research

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PD Dr. Daniel Burckhardt (Naturhistorisches Museum Basel)

Dr. Nicolas Sauvion (INRA Montpellier)

Dr. Phyllis Weintraub (Gilat Research Center, ARO Volcani Center)

Workshop Program

Monday, April 19th:

09:00 Welcome and presentation of the workshop program (B. Jarausch)

09:30 Presentation of participants

10:00 Introduction into taxonomy, systematics, biology, host plant relationships and biogeography of psyllids, part 1 (D. Burckhardt)

11:00 Coffee break

11:15 part 2 (D. Burckhardt)

13:00 Lunch

14:00 Practical identification of psyllids, 1. session

16:00 Coffee break

16:15 Practical identification of psyllids, 2. session

18:00 End of session

Tuesday, April 20th:

09:00 Welcome by the head of the institute (Gabi Krczal)

09:30 Visit of the insectarium of AlPlanta

10:30 Coffee break

11:00 Practical identification of psyllids, 3. session

13:00 Lunch

14:00 Practical identification of psyllids, 3. session

18:00 End of session

Wednesday, 21st:

09:00 Field excursion, trapping methods of psyllids

13:00 Pick nick in the experimental orchard of DLR Rheinpfalz

15:00 Practical identification of psyllids, 4. session

18:00 End of session

18:30 Guided visit to Neustadt / Weinstrasse

Thursday, 22nd:

09:00 Introduction into molecular analysis of psyllids as phytoplasma vectors (W. Jarausch, N. Sauvion)

09:30 Extraction of total DNA from psyllids

13:00 Lunch

14:00 Use of molecular markers for psyllid identification by PCR

16:00 Coffee break

16:30 Continue with molecular analysis

18:00 End of session

Friday, April 23rd:

09:00 Gel Elektrophoresis of PCR products and documentation

10:30 Coffee Break

11:00 Final discussion of results

12:00 Lunch

13:00 End of the workshop

I. part: Psyllid identification by morphological means

Glossary

aedeagus: penis
anal break: break in the vein along the posterior wing margin at the apex of the claval fold
antenna: usually 10-segmented
apical spurs: strongly sclerotised spurs at the metatibial apex (tibia of hind leg)
capitate seta: seta which is apically inflated
caudal plate: slerotised terminal plate of the nymphal abdominal dorsum
cell: surface between veins of wing
circumanal ring: ring consisting of densely spaced wax pores surrounding the nymphal anus
dorso-ventral: in the vertical body axis
dorsum: back
metabasitarsus: basal segment of tarsus of hind leg
metatarsus: tarsus of hind leg
metatibia: tibia of hind leg
ovipositor: female terminalia consisting of proctiger, subgenital plate and valvulae
paramere: paired structure attached to the male subgenital plate used during copulation to hold female terminalia
proctiger: abdominal tergite containing anus
sectaseta (plural sectasetae): short bipartite seta
seta (plural setae): bristle, hair
sternite: abdominal, ventral sclerite
subgenital plate: genital sternite
tergite: abdominal dorsal sclerite
valvula (plural valvulae): part of the female ovipositor used to lay the egg
venation: the forewing consists of a characteristic arrangement of the veins which separate the cells
venter: the ventral body surface
wing buds: nymphal structures from which the adult wings develop

Identification key for the Central European *Cacopsylla* species

Daniel Burckhardt, Naturhistorisches Museum Basel

1 Mesoscutum distinctly longer than mesopraescutum along median longitudinal body axis, the latter about twice as long as pronotum ..... subgenus ***Thamnopsylla*** 3

\- Mesoscutum about as long as or slightly longer than mesopraescutum; both more than twice as long as pronotum 2

2 Dorsal surface spinules in cell rs of forewing above bifurcation of vein M arranged in squares or rhombi of about 20 µ length; surface spinules in cell c+sc restricted to apical portion of cell or entirely reduced; surface spinules reduced in basal part of rs, at most a few spinules present; fields of surface spinules tapering along apical wing margin; forewing membrane always colourless; pterostigma oblong cuneate, evenly tapering. Antenna shorter than 1.75 mm, if longer then forewing longer than 3 mm. Male paramere simple, lamellar. Female terminalia short, cuneate ..... subgenus ***Cacopsylla*** s. str. 14

\- Forewing spinulation different, or wing membrane yellowish or brownish, or pterostigma elongate with subparallel margins. If antenna longer than 1.75 mm then forwing shorter than 3.0 mm. Male paramere often complex. Female terminalia different ..... subgenus ***Hepatopsylla*** 17

3 Dorsal surface spinules of forewing in cell rs above bifurcation of vein M very densely and irregularely spaced (2–10 µ) 4

\- Dorsal surface spinules of forewing in cell rs above bifurcation of vein M evenly spaced in 15–20 µ distance forming squares or rhombi 5

4 Forewing bearing dark ribbon apically. Metatibia without genual spine. Male subgenital plate bearing apical tubercular extension. Female proctiger and subgenital plate ending in thorn-like process ..... ***breviantennata***

\- Forewing irregulary dark without distinct apical ribbon. Metatibia with genual spine. Male subgenital plate rounded apically. Female proctiger and subgenital plate evenly tapering in profile ..... ***pruni***

5 Dorsal surface spinules covering entire cell c+sc of forewing apart from stripes along veins; forming extended fields in other cells which taper towards wing margin; membrane colourless or fumate but never with brown stripe along vein Cu_{1b} which is strongly contrasted from surroundings 6

\- Dorsal surface spinules of forewing more or less reduced, or not tapering towards wing margin, or wing pattern consisting with dark, strongly contrasted stripe along vein Cu_{1b} 9

6 Antenna usually longer than 1.2 mm. Genal processes broad and blunt. Paramere broad, lanceolate. Dorsal margin of female proctiger raised in the middle, apex rounded ..... ***pyrisuga***

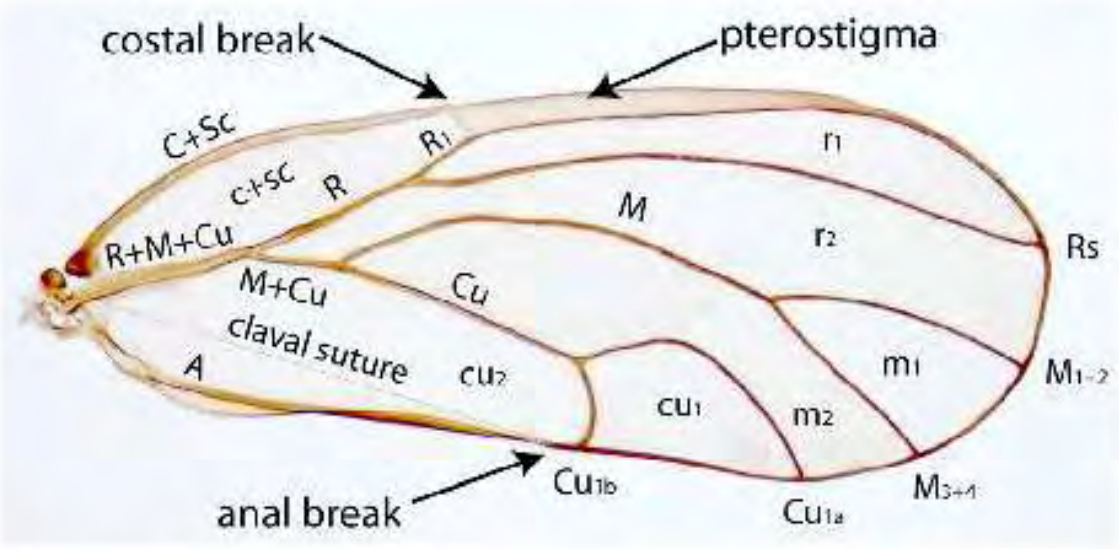
\- Antenna usually shorter than 1.1 mm. Paramere narrow or with apical processes. Dorsal margin of female proctiger concave in the middle, or apex angular 7

7 Paramere, in profile, with square base bearing 2 apical processes. Dorsal margin of female proctiger, distal of circumanal ring, evenly concave; apex rounded ..... ***picta***

\- Paramere, in profile, elongate; apex with inward and forward pointing tooth. Dorsal margin of female proctiger raised in the middle; apex angular 8

8 Paramere, in profile, narrowed in the middle. Apex of distal segment of aedeagus weakly curved ..... *melanoneura*
- Paramere, in profile, evenly tapering from base to apex. Apex of distal segment of aedeagus strongly curved, hook-shaped ..... *affinis*
9 Forewing membrane bearing dark brown patch along vein Cu_{1b} 10
- Forewing membrane along vein Cu_{1b} of same colour as surrounding membrane 13
10 Forewing with dark, continuous ribbon along apex ..... *limbata*
- Forewing lacking dark, continuous ribbon along apex 11
11 Forewing bearing dark brown patches on tips of veins, at the bifurcation of vein M, and in the middle of vein Cu_{1a} ..... *pulchella*
- Forewing pattern different 12
12 Areas of radular spinules of cells m_1, m_2 and cu_1 of forewing more or less dark; dark patch along vein Cu_{1b} reaching bifurcation of Cu, straight in proximal half; vein Cu_{1a} angular; surface spinules reduced in cells c+sc and r_1 ..... *crataegi*
- Areas of radular spinules of cells m_1, m_2 and cu_1 of forewing light; dark patch along vein Cu_{1b} not reaching bifurcation of Cu, not narrowed in proximal half; vein Cu_{1a} rounded; surface spinules forming extended fields in cells c+sc and r_1 ..... *albipes*
13 Surface spinules of forewing forming very narrow fields. Antenna longer than 1.3 mm ..... *rhamnicola*
- Surface spinules of forewing forming extended fields. Antenna shorter than 0.9 mm ..... *corcontum*
14 Antenna shorter than 1.2 mm ..... *peregrina*
- Antenna longer than 1.3 mm 15
15 Antenna longer than 1.75 mm ..... *ulmi*
- Antenna shorter than als 1.55 mm 16
16 Antennal segments 4–8 with black apex ..... *sorbi*
- Antennal segments 4–8 with yellow or ochreous apex ..... *mali*
17 Surface spinules of forewing forming more or less even squares or rhombi of 20 µ distance in cell rs above bifurcation of vein M; fields of surface spinules tapering towards wing margin. Clavus with brown apex 18
- Character combination different 20
18 Paramere sickle-shaped. Female proctiger strongly narrowed in the middle ..... *pyri*
- Paramere lamellar. Female proctiger cuneate 19
19 Genal processes blunt. Paramere bearing two apical teeth, one long, forwards directed and one short, inwards directed; foremargin with wide lobe. Distal segment of aedeagus with very wide, weakly curved apical dilatation. Dorsal margin of female proctiger with small swelling in the middle, apex blunt ..... *bidens*
- Genal processes subacute. Paramere bearing one blunt, inwards directed apical tooth; foremargin more or less straight. Distal segment of aedeagus with very wide, hook-shaped apical dilatation. Dorsal margin of female proctiger concave ..... *pyricola*
20 Dorsal surface spinules of forewing in cell rs above bifurcation of vein M irregularly, densely spaced (2–10 µ) or arranged in transverse rows 21
- Dorsal surface spinules of forewing forming more or less even squares or rhombi of 20 µ distance in cell rs above bifurcation of vein M 27
21 Surface spinules arranged in transverse rows 22
- Surface spinules densely, irregularly spaced 24

22	Paramere, in profile, with large basal lobe. Dorsal margin of female proctiger straight or weakly convex	*elegantula*
-	Paramere, in profile, lamellar with anteriorly directed apical tooth. Dorsal margin of female proctiger sinuous	23
23	Thorax brown, abdomen green, Terminalia ochreous or brown	*abdominalis*
-	Body colour evenly light or reddish brown	*intermedia*
24	Forewing in cell c+sc without ventral surface spinules	25
-	Ventral surface spinules present in cell c+sc	26
25	Forewing oval, widest in the middle; wing apex near apex of vein M_{1+2}. Antenna shorter than 1.0 mm	*parvipennis*
-	Forewing widest in apical third; wing apex at the middle of outer margin of cell rs. Antenna longer than 1.0 mm	*flori*
26	Dorsal surface spinules of forewing light, leaving spinule-free stripes along the veins; cell c+sc entirely covered in ventral surface spinules	*ambigua*
-	Dorsal surface spinules of forewing dark, covering the entire membrane up to the veins; ventral surface spinules present only in distal part of cell c+sc	*propinqua*
27	Antenna longer than 1.6 mm	28
-	Antenna shorter than 1.3 mm	32
28	Metatibia with 1+1+(2–3)+1 sclerotised apical spurs	29
-	Metatibia with 1+3+1 sclerotised apical spurs	30
29	Antennal segments 3–7 yellowish or ochreous with dark brown apex. Fields of surface spinules tapering along apical wing margin	*visci*
-	Antennal segments 3–7 entirely yellow or ochreous. Fields of surface spinules evenly widening towards apical wing margin	*viburni*
30	Forewing brown to dark brown in apical half with colourless window in cell cu_1	*fulguralis*
-	Forewing light or brown but lacking contrasted colourless window in cell cu_1	31
31	Body colour dark brown. Paramere with short, angular apical, sclerotised apex. Valvula 2 of female terminalia with straight ventral margin	*zetterstedti*
-	Body colour green or yellow. Paramere with long, curved apical, sclerotised apex. Valvula 2 of female terminalia with concave ventral margin	*hippophaes*
32	Pterostigma cuneate, broad and short, with converging margins ending in the middle of vein Rs; wing membrane yellowish or ochreous, veins ochreous or light brown	33
-	Pterostigma long and narrow, with subparallel margins ending in apical third of vein Rs; wing membrane colourless or dark, veins light or dark	35
33	Foremargin of forewing relatively straight	*rhododendri*
-	Foremargin of forewing strongly curved	34
34	Surface spinules entirely covering cell c+sc of forewing	*myrtilli*
-	Surface spinules absent from basal third of cell c+sc of forewing	*ledi*
35	Male paramere bearing subapical lobe along hind margin	36
-	Male paramere lacking subapical lobe along hind margin	38
36	Paramere lacking basal lobe at hind margin	*moscovita*
-	Paramere bearing basal lobe at hind margin	37
37	Basal lobe at hind margin of paramere not incised dorsally	*saliceti*
-	Basal lobe at hind margin of paramere strongly incised dorsally	*iteophila*
38	Apex of paramere forming simple, backwards directed sclerotised tooth	*pulchra*
-	Apex of paramere with two strongly sclerotised teeth	39
39	Paramere, in rear view, with a tooth in apical third	*brunneipennis*
-	Paramere, in rear view, with lobe in the middle of inner margin	*nigrita*



Nomenclature of forewing veins and cells.

Cacopsylla picta – vector of *Ca.* Phytoplasma mali

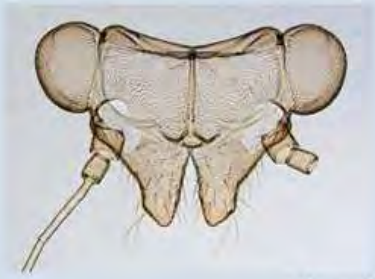
Name: *Cacopsylla picta* (Foerster, 1848)
Synonyms: *Psylla picta; Cacopsylla (Thamnopsylla) picta; Psylla costalis* Flor, 1861; *Psylla nobilis* Meyer-Dür, 1871; *Psylla pyrastri* Löw, 1876; *Psylla chlorostigma* Löw, 1886
Distribution: Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Italy, Lithuania, Moldava, Poland, Russia, Slovakia, Sweden, Switzerland, Turkey, Ukraine, United Kingdom
Host plants: *Malus domestica, M. sylvestris, Prunus armeniaca*

nymph

adult (male)

➡ paramere

➡ distal portion of aedeagus



head

forewing

male terminalia

female terminalia

Cacopsylla melanoneura – vector of *Ca.* Phytoplasma mali

Name: *Cacopsylla melanoneura* (Foerster, 1848)
Synonyms: *Psylla melanoneura; Cacopsylla (Thamnopsylla) melanoneura; Psylla crataegi sensu* Foerster, 1848, *nec* Schrank, 1801; *Psylla pityophila* Flor, 1861; *Psylla oxyacanthae* Meyer-Dür, 1871; *Psylla similis* Meyer-Dür, 1981
Distribution: Armenia, Austria, Azerbajan, Belgium, Bulgaria, China, Croatia, Czech Republic, Denmark, France, Georgia, Germany, Greece, Ireland, Italy, Moldova, Mongolia, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Tajikistan, Ukraine, United Kingdom, Uzbekistan
Host plants: *Crataegus* species, *Malus domestica, Mespilus germanica, Pyrus communis*

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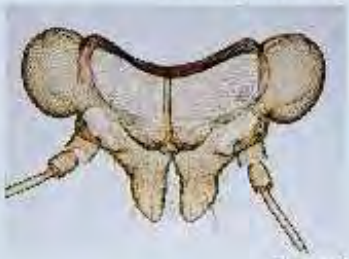
nymph

adult (female)



head

paramere

distal portion of aedeagus

forewing

male terminalia

female terminalia

Cacopsylla pruni – vector of *Ca.* Phytoplasma prunorum

Name: *Cacopsylla pruni* (Scopoli, 1763)
Synonyms: *Chermes pruni; Psylla pruni; Cacopsylla (Thamnopsylla) pruni; Psylla fumipennis* Foerster, 1848
Distribution: Armenia, Austria, Azerbajan, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Georgia, Germany, Iran, Ireland, Italy, Moldova, Norway, Poland, Romania, Russia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom
Host plants: *Prunus* species

nymph

adult (female)

head

paramere

distal portion of aedeagus

forewing

male terminalia

female terminalia

Cacopsylla pyri – vector of *Ca.* Phytoplasma pyri

Name: *Cacopsylla pyri* (Linnaeus, 1761)
Synonyms: *Chermes pyri; Psylla pyri; Cacopsylla (Hepatopsylla) pyri; Psylla pyrarboris* Šulc, 1910
Distribution: Armenia, Austria, Azerbajan, Belgium, China, Czech Republic, Denmark, Finland, France, Georgia, Germany, Greece, Italy, Kazakhstan, Malta, Moldova, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom
Host plants: *Pyrus communis, P. elaeagnifolia*

nymph

adult (female)

head

paramere

distal portion of aedeagus

forewing

male terminalia

female terminalia

Cacopsylla pyricola – vector of *Ca.* Phytoplasma pyri

Name: *Cacopsylla pyricola* (Foerster, 1848)
Synonyms: *Psylla pyricola; Cacopsylla (Hepatopsylla) pyricola; Psylla apiophila Foerster, 1848; Psylla argyrostigma* Foerster, 1848; *Psylla simulans* Foerster, 1848; *Psylla horvathi* Šulc, 1913; *Psylla vicina* Šulc, 1915
Distribution: Austria, Belgium, Croatia, Czech Republic, Denmark, France, Germany, Greece, Italy, Moldova, Netherlands, Norway, Poland, Romania, Slovakia, Spain, Sweden, Switzerland, Ukraine, United Kingdom; Introduced into the USA, Canada
Host plants: *Pyrus communis, P. pyraster*

nymph

adult (male)

paramere

head

distal portion of aedeagus

forewing

male terminalia

female terminalia

II. part: Psyllid identification by molecular means

Development of specific primers for the molecular identification of *Cacopsylla picta*, the main vector of apple proliferation disease

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Cacopsylla picta has been shown to be the main vector of apple proliferation disease. However, identification of this psyllid by morphological means is difficult for less experienced persons. On the other hand, molecular screening for phytoplasma infection in insects has become of increasing importance to identify the vectors of AP and to analyse the disease spread in different apple growing regions. Therefore, molecular markers for the identification of *C. picta* were developed. At the beginning of this study, DNA nucleotide sequence data of psyllids were only available for one genetic locus, the wingless (*wg*) gene. Based on these sequence data primers were selected which amplified a wide range of *Cacopsylla* species. So far, sequence data of a fragment of the *wg* gene were produced for 23 different psyllid species, including the known phytoplasma vector species *C. picta*, *C. melanoneura*, *C. pruni*, *C. pyri* and *C. pyricola*. The sequence comparison enabled the development of specific primers for *C. picta*. The specificity of the primers was tested for a range of more than 40 psyllid species, predominantly those which are known to occur in apple and stone fruit orchards in Central Europe. The universality of the primers was tested for *C. picta* samples originating from 33 different locations in Germany, France, Italy, Czech Republic and Switzerland. Furthermore, the available sequence data were used to establish a first phylogenetic tree of psyllid species based on the *wg* locus.

Protocol for extraction of total DNA from insects with a modified CTAB method

(W. Jarausch, AlPlanta-IPR, RLP AgroScience, Neutstadt/W. Germany)

reagent	1 psyllid	3 psyllids	5 psyllids	10 psyllids
TexDIR-buffer	100 µl	150 µl	200 µl	250 µl
CIA	100 µl	150 µl	200 µl	250 µl
EtOH abs.	250 µl	300 µl	400 µl	500 µl
70% EtOH	500 µl	500 µl	500 µl	500 µl
H2O	25 µl	25 µl	50 µl	50 µl

- place insects into a 1,5 ml-Eppendorf tubes
- add TEXDir-buffer (volume adjusted to the amount of individuals, see table)
- homogenise with a sterile micropistill
- incubate at 65°C in water bath for 30 min
- add CIA 24:1 according to the volume indicated in the table
- mix thouroughly
- centrifugation: 5 min, 13000 rpm
- transfer supernatant to a sterile 1,5 ml-Eppendorf tube
- add pre-cooled EtOH abs. according to the volume indicated in the table
- DNA precipitation: -80°C, 45 min
- centrifugation: 15 min, 13000 rpm, 4°C
- discard supernatant
- wash pellet with 500µl 70% EtOH
- centrifugation: 10 min, 13000 rpm, 4°C
- discard supernatant
- dry pellet in speedvac
- dissolve in 25 or 50µl H_2O
- short term storage at 4°C, long term storage at -20°C

TEXdir-buffer (for 1L)

20 g CTAB
20 g PVP25
82 g NaCl
dissolve in 500 mL H_2O dest. by heating and constant stirring

add: 40 mL 0,5 M EDTA (pH 8,0)
100 mL 1 M TrisHCl (pH 8,0)
adjust to 1 L with H_2O

CIA for 500 mL

480 mL chloroform
20 mL isoamylaclohol

Universal PCR set up

Mix for 1 reaction:	**Mix for 16 reactions:**
0,5 µl dNTPs (5mM each = 20mM) 0,2 µl forward primer 1 (100µM) 0,2 µl reverse primer 2 (100µM) 2,0 µl 10x Taq-Puffer (incl. Mg^{2+}) **17 µl** H_2O dest. **0,1 µl Taq-DNA-polymerase (5 U/µl)** total volume: 20 µl place 19 µl in PCR tube + 1,0 µl genomic DNA	8 µl dNTPs (5mM each = 20mM) 3,2 µl primer 1 (100µM) 3,2 µl primer 2 (100µM) 32 µl 10x Taq-Puffer (incl. Mg^{2+}) **272 µl** H_2O dest. **1,6 µl Taq-DNA-polymerase (5 U/µl)** total volumen 320 µl place 19 µl in PCR tube + 1,0 µl genomic DNA

Participants

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Psyllid Training School Neustadt / W, Germany, 19th-23rd April 2010