A survey of potential insect vectors of mountain pine proliferation decline phytoplasma in Curonian Spit, Lithuania.

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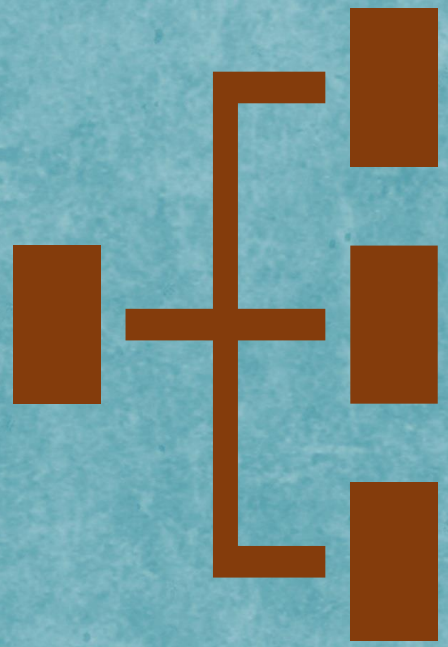
Phytoplasmas:

For a long time researched as plant viruses that caused plant yellowing diseases. Discovered in 1967 by Doi *et*. all.

Plant pathogenic bacteria
Reside in plant phloem and insect-host tissues
200-800 nm cell size
530-1350 kbp size genomes
Wall-less, polymorphic
Gram positive
Unculturable in artificial media

http://1.bp.blogspot.com/-63aX2TQr3AY/UNEcJHexG2I/AAAAAAAAAqQ/GCejz-JbLV0/s1600/TEM_Phytoplasmas_Jana_Franova.jpg

Phytoplasma taxonomy



- Taxonomy is based on marker gene analysis: mainly of 16S rRNA gene
- Phytoplasmas belong to the phylum Firmicutes, class Mollicutes
- Phytoplasmas as unculturable microorganisms received '*Candidatus* (*Ca*.)Phytoplasma' species status, that was given by the International Research Programme for Comparative Mycoplasmology in 2004
- 44 '*Ca*. Phytoplasma' species are confirmed to date [1]

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Phytoplasma dissemination

- Phytoplasmas in nature are spread by insects: mainly from the order Hemiptera (leafhoppers, planthoppers, psyllids)
- Also they can be transmitted by dodder (parasitic plant) and anthropogenically (grafting, germplasm)

Leafhopper

Dodder

Grafted pine tree

Phytoplasma research methods

- Molecular biology methods: PCR, RFLP, hybridization, qPCR, NGS, etc.
- Immunological: staining (DAPI, Diene’s stain), ELISA
- Electron microscopy

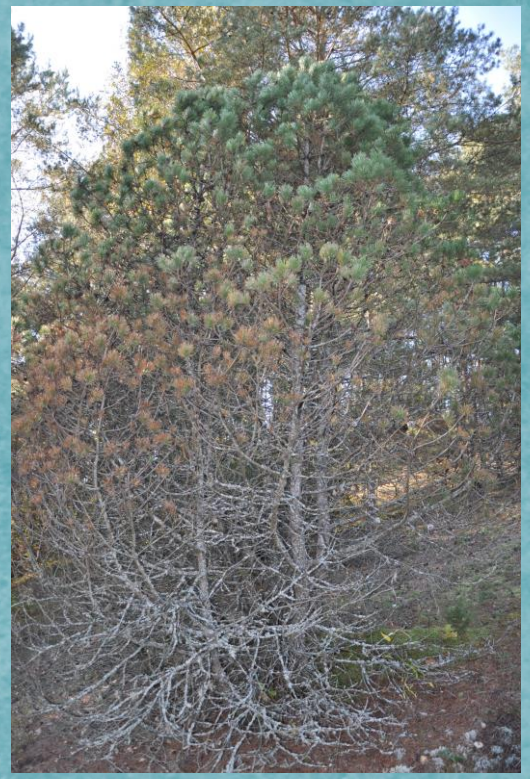
Economical impact

Phytoplasmas are known to infect more than 1000 of plant species worldwide.

They are causing epidemics of the agricultural and the industrial plants, thus, they inflict a meaningful impact on economics.

Infected strawberry

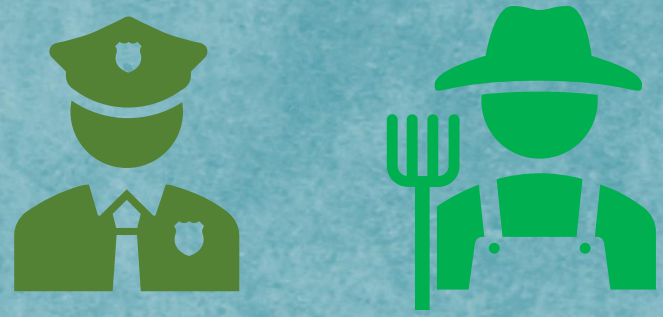
Infected common oaks



Infected Scots pine

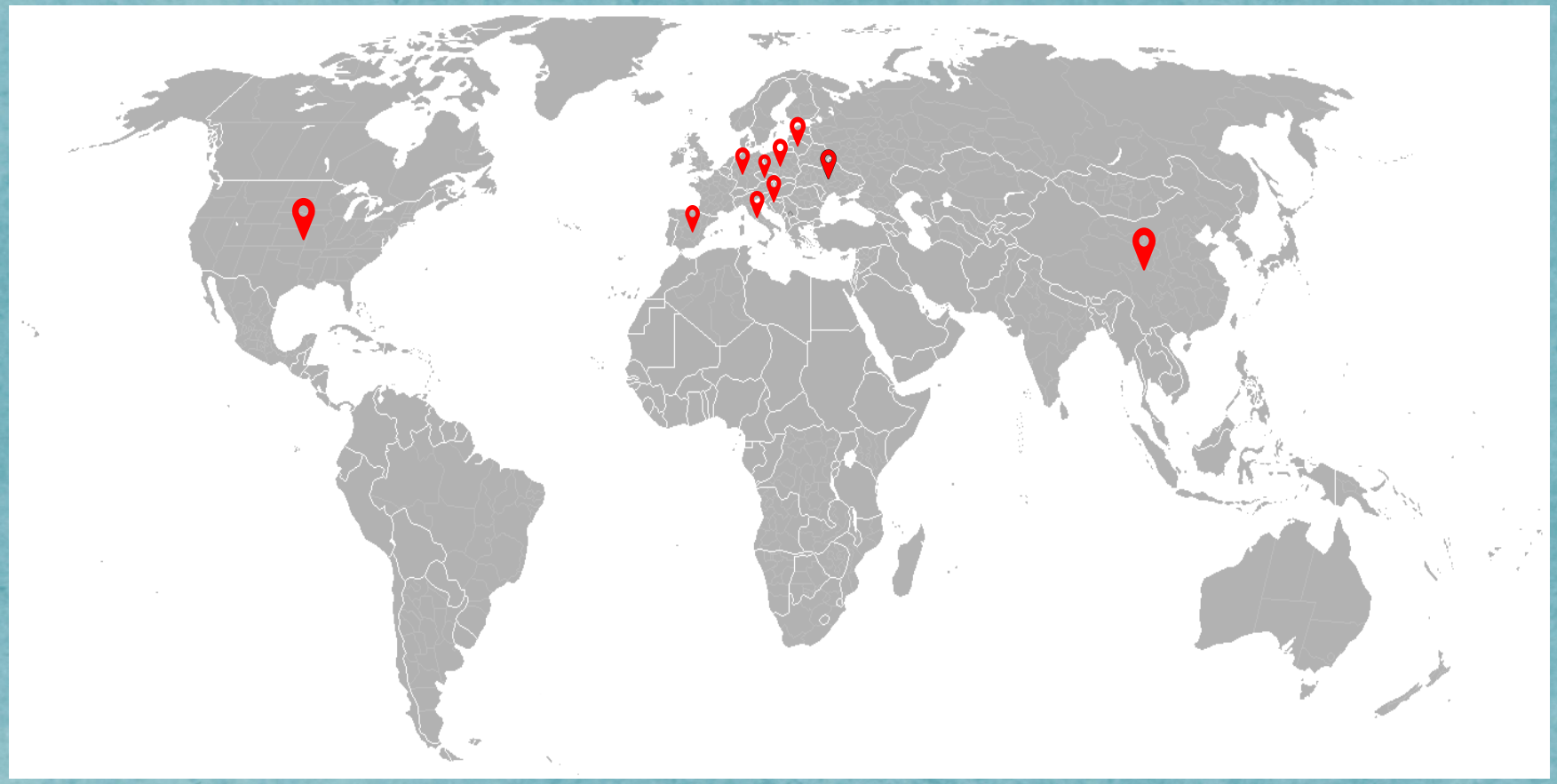
Control of the phytoplasmas

- Strict quarantine measures
- Agrotechnical methods (physical, biological fencing, application of insect repellents, insecticides, disposal of infected plants , etc.)
- Germplasm thermotherapy
- Antibiotic treatment of plants (tetracycline)



Phytoplasmas infecting gymnosperms

- Pine [2, 5, 12]
- Spruce [2, 3]
- Cycad [7, 8]
- Larch [6]
- Juniper [4]
- Cypress [9]



https://commons.wikimedia.org/wiki/File:BlankMap-World-2009.PNG

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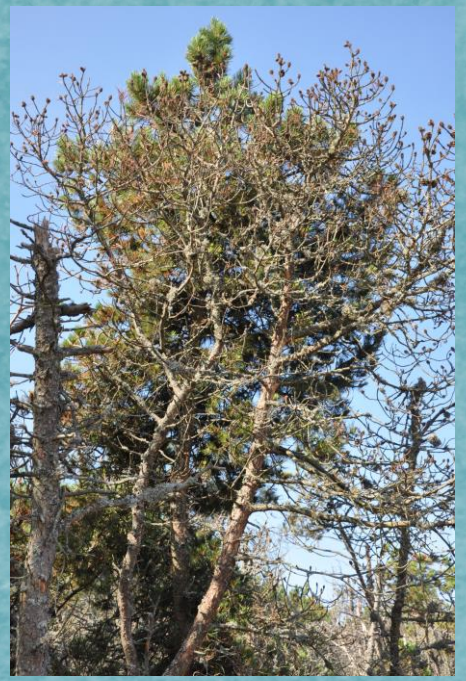
Conifers important industrial plants

- The wood of the conifer trees, is an economically important Lithuanian and international export commodity, and is highly valued in the industry
- Conifers are an important item for the landscaping and the gardening
- Phytoplasma infection damages trees and can make them vulnerable to other pathogens and climatic stress

https://www.google.com/url?sa=i&url=https%3A%2F%2Fsavvyrest.com%2Fblog%2Fwhy-sustainability-important-timber-industry&psig=AOvVaw2xYv3nM-aixk0CUol3OOPs&ust=1603314859545000&source=images&cd=vfe&ved=0CAIQjRxqFwoTCLi-mbiLxOwCFQAAAAAdAAAAABAD

https://www.google.com/url?sa=i&url=https%3A%2F%2Fgardentabs.com%2Fpine-tree-landscaping%2F&psig=AOvVaw3CCWWi4JHCkYmJLFDr3ylO&ust=1603314803730000&source=images&cd=vfe&ved=0CAIQjRxqFwoTCJC32JyLxOwCFQAAAAAdAAAAABAE



Phytoplasma infected mountain pine

‘*Ca*. Phytoplasma pini’ in Europe

Croatia [12]
Czech Republic [11]
Germany [10]
Lithuania [2, 12]
Poland [11]
Spain [10]

Insect-vector unknown!

www.freeworldmaps.net

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‘*Ca*. Phytoplasma pini’ in Lithuania

‘*Ca*. Phytoplasma pini’ was detected in Scots pine (*Pinus sylvestris* L.) [13] and mountain pine (*Pinus mugo* Turra) [2] in Lithuania

https://pt.wikipedia.org/wiki/Transporte_ferrovi%C3%A1rio_na_Litu%C3%A2nia#/media/Ficheiro:Un-lithuania.png

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The infestation magnitude of the Curonian Spit mountain pines (*P. mugo*) infected with '*Ca*. Phytoplasma pini' phytoplasmas can be as high as 80% [2]

Goal

- Our goal was to survey mountain pines of the Curonian Spit for the insects-hosts of mountain pine proliferation decline (MPPD) phytoplasma.

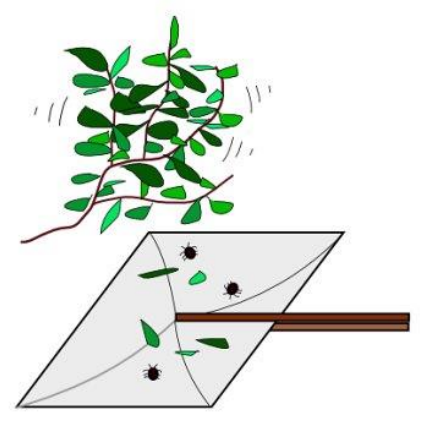
Diseased mountain pines (*P. mugo*)

Symptomatic mountain pine trees exhibiting symptoms of dwarfed needles, proliferation, decline, infected with MPPD phytoplasma

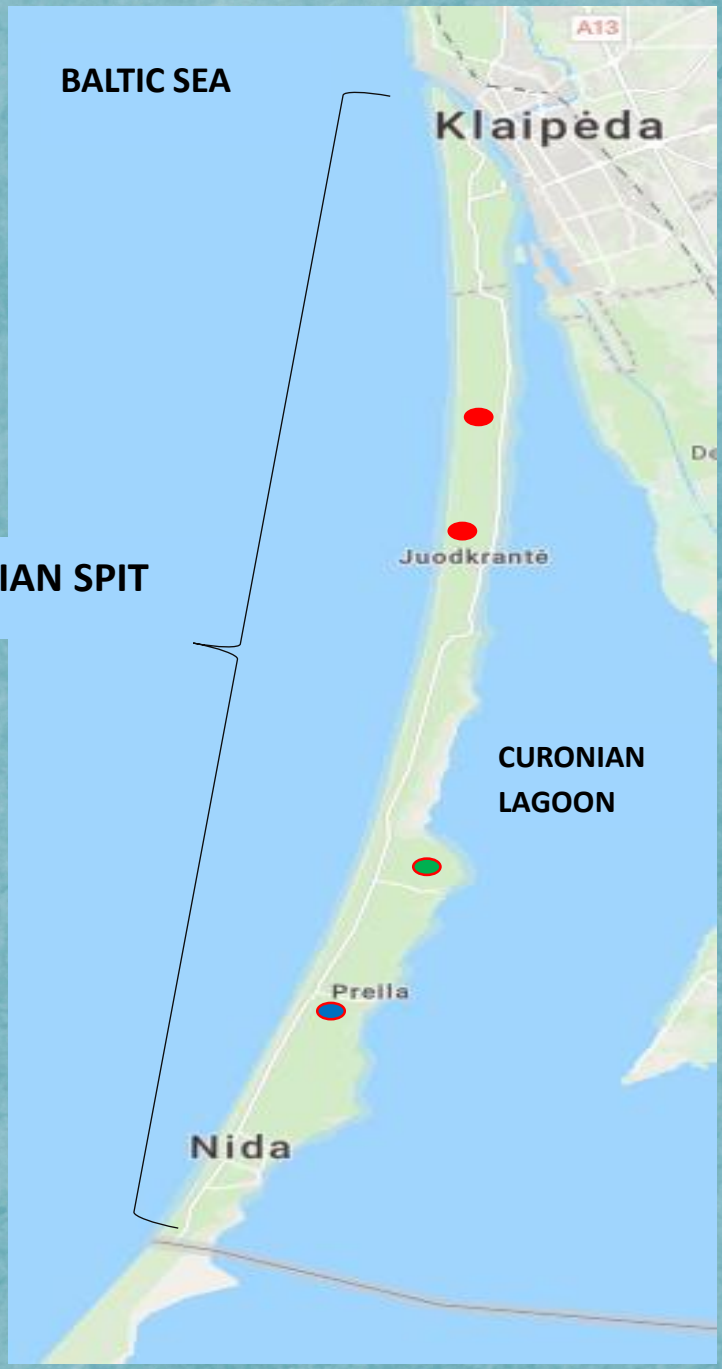
Insect collection locations

Juodkrante
Preila
Pervalka

Collected using beating tray method from the symptomatic mountain pines.



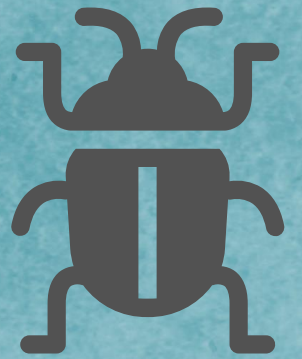
https://www.amentsoc.org/insects/glossary/terms/beating-tray

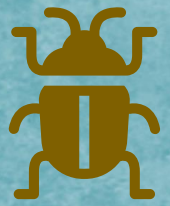


Collected insects

Order Coleoptera
Family Carabidae
Calodromius spilotus Ill.
Family Curculionidae
Brachyderes incanus L.
Pissodes pini L.
Pissodes validirostis Gyll.
Strophosoma capitatum (De Geer, 1775)
Pissodes piceae (Illiger, 1807)
Pissodes piniphilus (Herbst, 1797)
Family Ptinidae
Ptinus subpilosus Strm.

Order Hemiptera
Family Myridae
Lygus rugulipennis Poppius, 1911
Family Lygaeidae
Gastrodes (Gastrodes) grossipes DeGeer, 1773
Suborder Sternorrhyncha
Family Aphididae
Anoecia (Anoecia) corni (Fabricius, 1775)
Cinara sp.
Cinara (Cinara) pini (Linnaeus, 1758)
Cinara (Schizolachnus) pineti (Fabricius, 1781)
Cinara (Cinara) piniphila (Ratzeburg, 1844)
Cinara (Cinara) pinihabitans (Mordvilko, 1895)
Cinara (Cinara) pinea (Mordvilko, 1895)





Detection and identification of phytoplasmas

- For this work we have collected and tested more than 1000 insect samples
- Phytoplasma 16S rDNA amplicons were amplified from samples of: *Cinara* sp., *Cinara* (*Cinara*) *pini*, *Cinara* (*Schizolachnus*) *pineti*, *Cinara* (*Cinara*) *piniphila*
- 1200 bp size 16S rDNA amplicons from infected mountain pines and aphids were sequenced and used in the virtual restriction fragment length analysis (RFLP), and for RFLP group affiliation using *i*PhyClassifier online tool

'*Ca*. Phytoplasma pini' insects-hosts

(A)

(B)

(C)

(A) *Cinara* (*Cinara*) *pini*

(B) *Cinara* (*Schizolachnus*) *pineti*

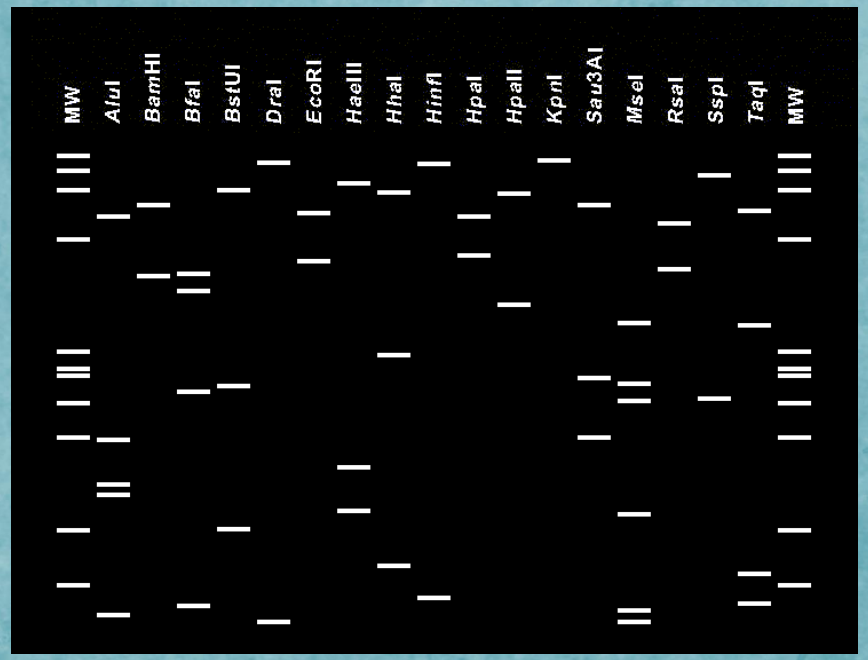
(C) *Cinara* (*Cinara*) *piniphila*

Photos by Danilovas J.

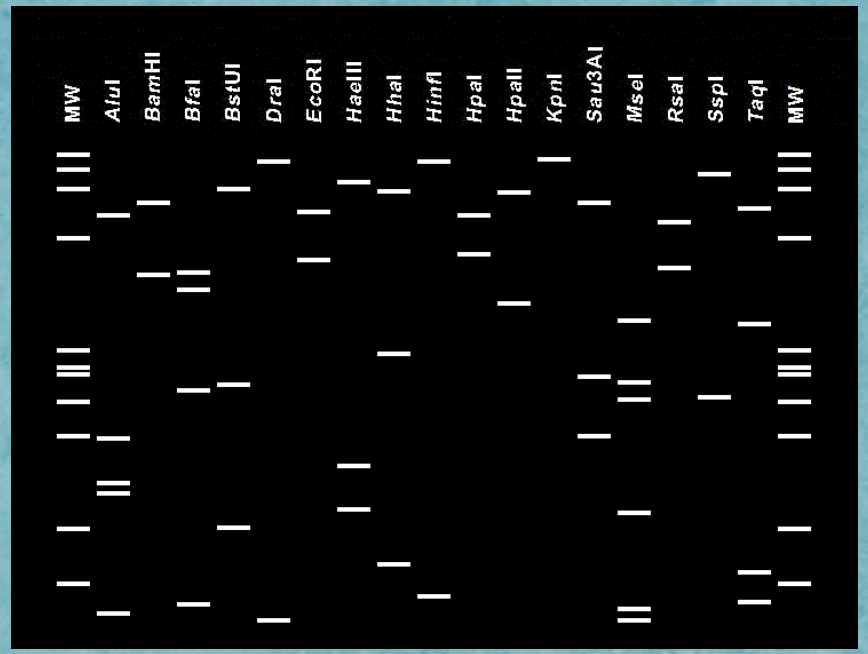
Virtual RFLP

Identification of MPPD phytoplasma 16SrXXI-A phytoplasma subgroup strain based on virtual RFLP analysis of 1.2 kb of 16S rDNA sequence.
16S rDNA amplicons were derived from *C. (C.) pini* and *P. mugo* samples.
MW – DNA size marker Φx174/*Hae*III

*i*PhyClassifier affiliation:
The phytoplasmas detected in *Cinara* sp., *Cinara* (*Cinara*) *pini*, *Cinara* (*Schizolachnus*) *pineti*, *Cinara* (*Cinara*) *piniphila* and *P. mugo* samples are variants of 16SrXXI-A phytoplasma subgroup



C. (*C.*) *pini*



P. mugo

Conclusions

- '*Ca*. Phytoplasma pini' (16SrXXI-A phytoplasma subgroup) for the first time was found in *C*. (*C*.) *pini*, *C*. (*C*.) *piniphila* and *C*. (*S*.) *pineti* insects in Lithuania and worldwide.
- RFLP analysis showed that the PCR-RFLP profile of the positive insect samples was consistent with that of the '*Ca*. Phytoplasma pini' from infected pine trees. These results suggest that *C*. (*C*.) *pini*, *C*. (*C*.) *piniphila* and *C*. (*S*.) *pineti* may be potential insect vectors of MPPD phytoplasma.

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Thank you

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