



Nederlandse Voedsel- en  
Warenautoriteit  
*Ministerie van Landbouw, Visserij,  
Voedselzekerheid en Natuur*

# Identification and characterization of novel *Neocosmospora* species following bark beetle outbreaks in the Netherlands

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EPPO Heads of Lab workshop, Estonia  
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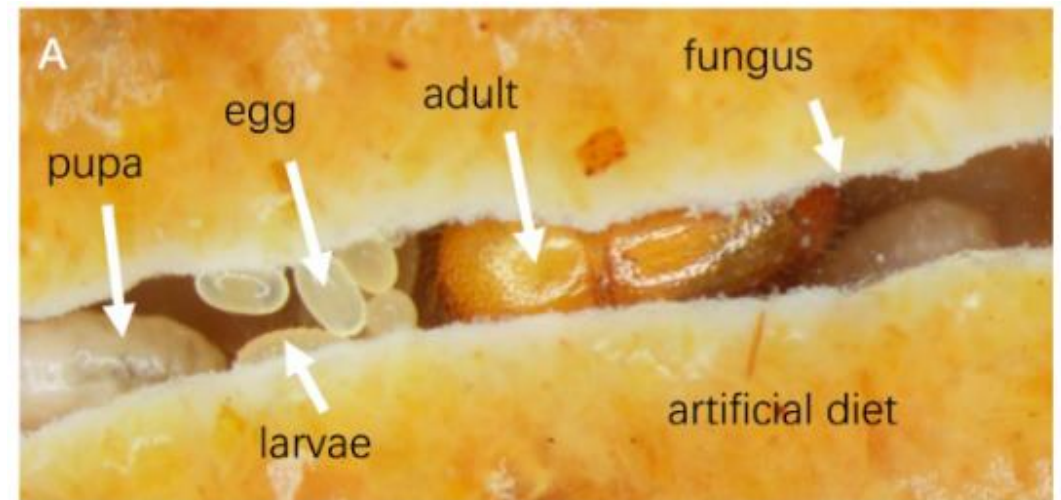


# The polyphagous shot hole borer (PSHB)

- › *Euwallacea fornicatus sensu stricto*
- › Colonize healthy trees and cause major damage
- › Create simple galleries in tree stems
- › Obligate symbiont with fungi from the genus *Neocosmospora*
- › Fungi grow in the galleries and serve as food for beetle larvae
- › Both *E. fornicatus s.s.* and the associated fungus (*N. euwallaceae*) are regulated



**Figure 1.** Adult *Euwallacea fornicatus* (Eichhoff). A-B female, C-D male. Photographs A and B by [Jiri Hulcr](#), University of Florida; C and D by [You Li](#), University of Florida.







# Outbreaks in Dutch greenhouses

- › Tropical trees intended for commercial wholesale
- › Beetles were found in three different greenhouses
- › Discoloration of the wood indicates presence of the fungus





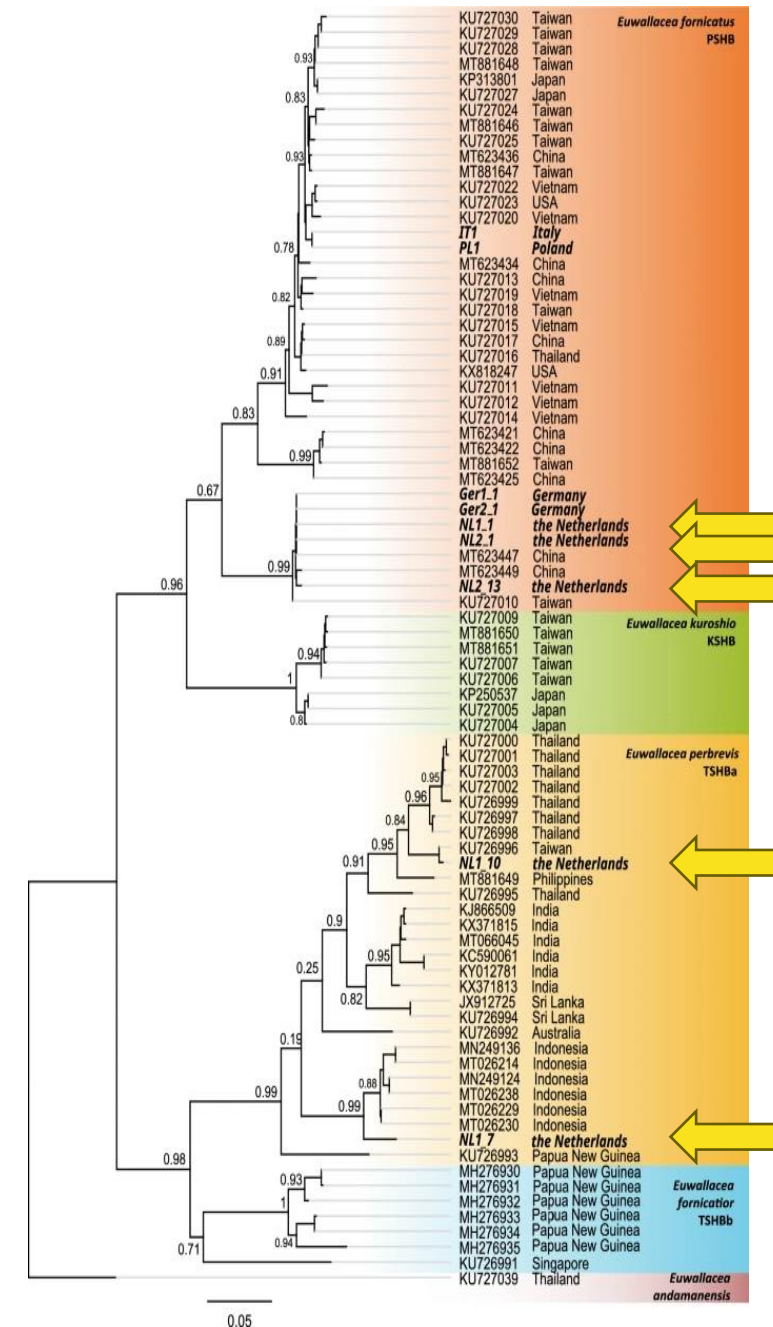
# *Beetle specimens*

- › Which species are present?
- › Are outbreak populations linked?



# Several *Euwallacea* species identified

- DNA barcoding: partial mtDNA *cox1* gene
- *Euwallacea fornicatus* s.s.
- *Euwallacea perbrevis*
- *Euwallacea* sp.
- Within species diversity suggests multiple original sources in individual greenhouses



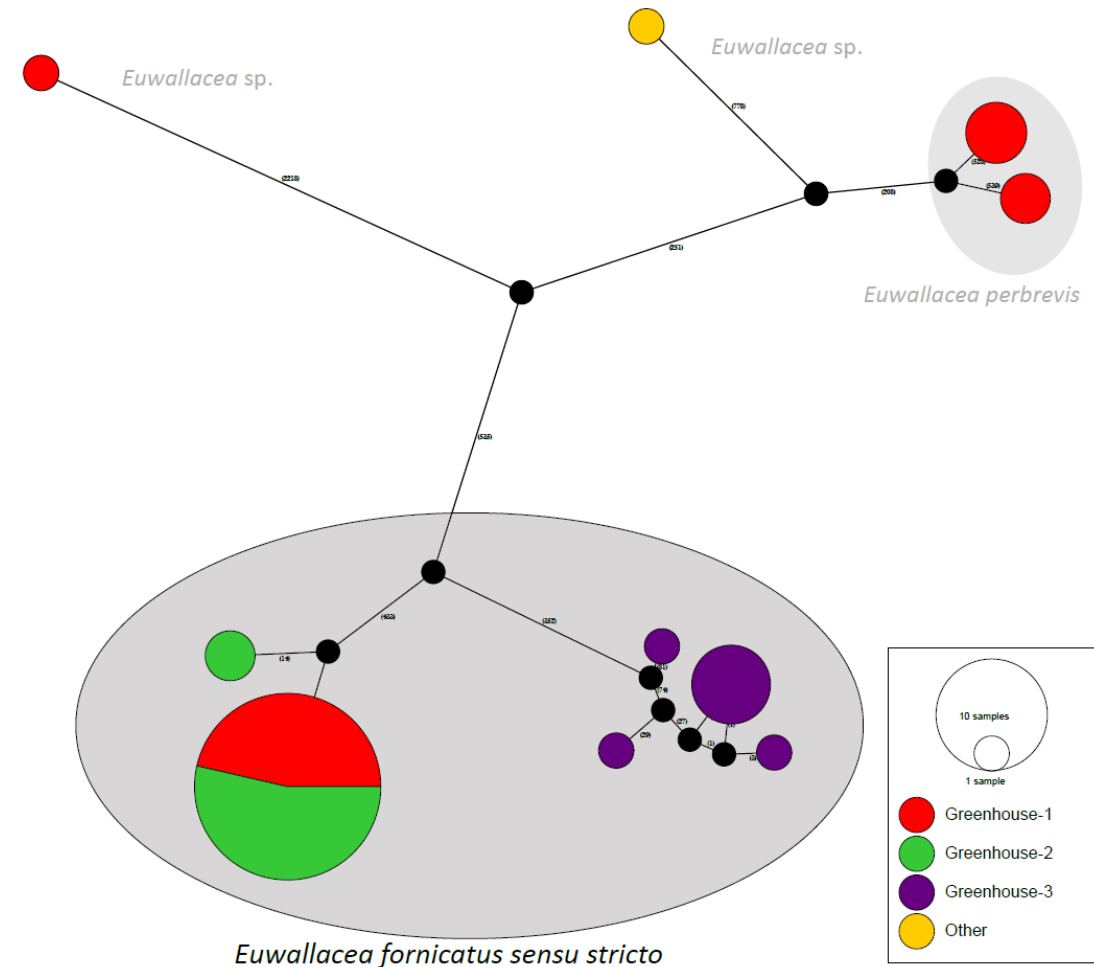






# Tracing outbreaks using complete mtDNA genomes

- > Two main groups within *E. fornicatus* s.s.
- > Outbreak populations in greenhouses 1 and 2 are linked (potential shared origin)
- > Greenhouse 3 is not linked to 1 and 2
- > No links to center of origin not possible due to lack of material





# *Fungal strains*

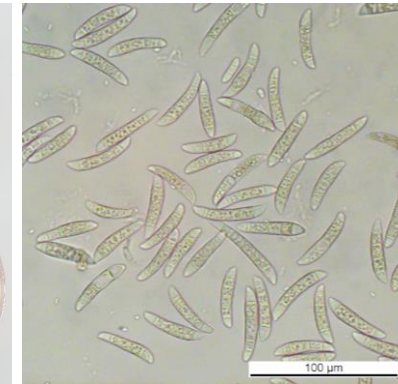
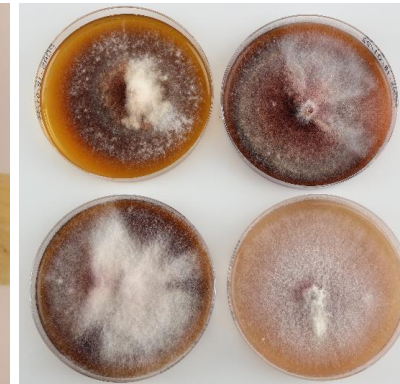
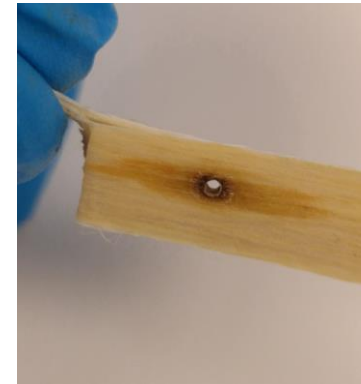
- › Is the associated regulated species present?
- › What is the biological relevance of novel species?





# Fungal cultures

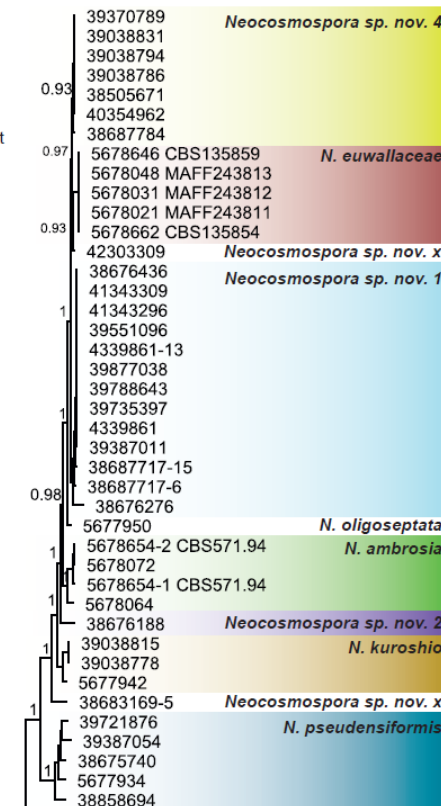
- Fungi were cultured from galleries and beetle heads (mycangia)
- Micro and macro-morphological characteristics
- Multi-locus sequence analysis
- Regulated *Neocosmospora* species (*N. euwallaceae* and *N. ambrosia*) not detected
- Data suggests presence of novel *Neocosmospora* species



**Five gene phylogeny of 57 *Neocosmospora* isolates**  
Single copy genes EF1a, rpb2, TUB and ACT and the ITS region (ITS1, 5.8s, ITS2) were extracted from *de novo* assembled whole genome shotgun data. A FastTree (approximately-maximum-likelihood, GTR, 100 bootstraps) was created from the obtained 10,099 nt (including gaps) MAFFT alignment.



Sandoval-Denis et al. 2019, Fig 1 detail: Maximum-Likelihood tree (ML) obtained from ITS, LSU, tef1 and rpb2 sequences of 376 strains from *Neocosmospora* species.



Neocosmospora Ambrosia clade (sensu Sandoval-Denis et al. 2019)

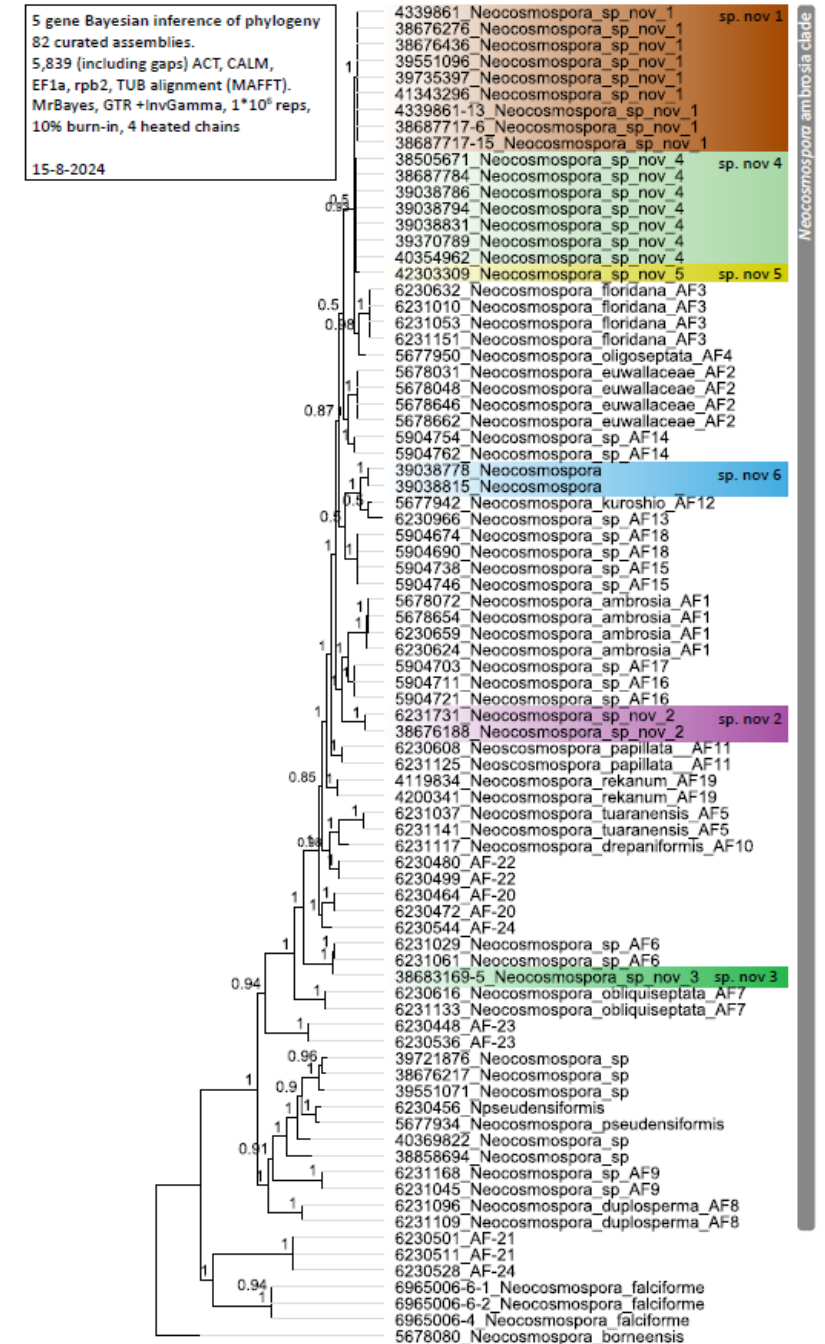




# Species description

- Description of six novel species is well supported by:
  - Micro and macro-morphological features
  - Optimal growth temperatures
  - MLSA and full genome phylogenomics

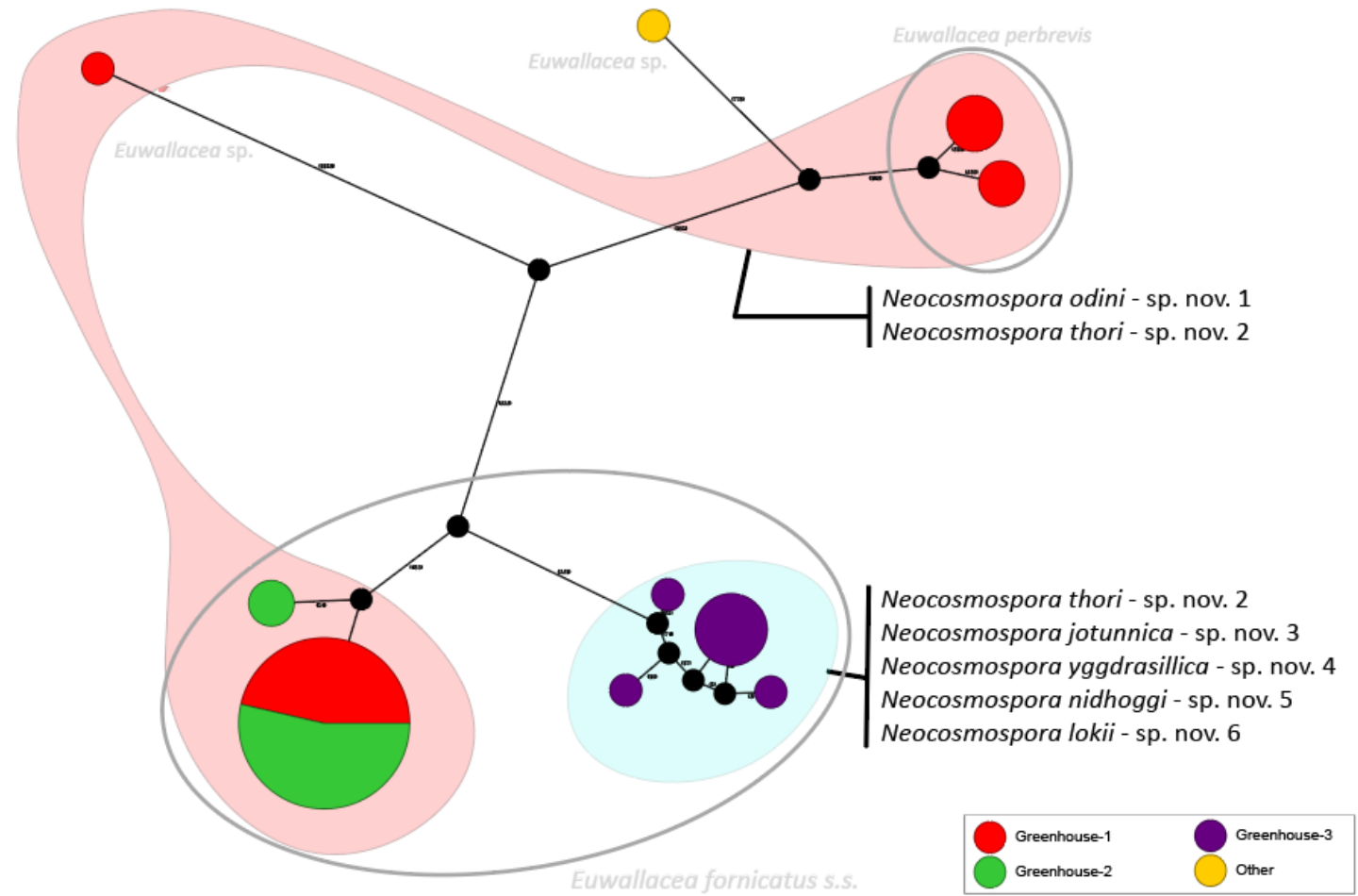
Species	Scientific Name	Mythological Inspiration
<i>Neocosmospora</i> sp. nov 1	<i>Neocosmospora odini</i>	Odin, the Allfather of the Norse gods, the first
<i>Neocosmospora</i> sp. nov 2	<i>Neocosmospora thori</i>	Thor, the god of thunder and strength, strong but stocky build
<i>Neocosmospora</i> sp. nov 3	<i>Neocosmospora jotunnica</i>	The Jötnar (giants) of Norse mythology, long as are the spores
<i>Neocosmospora</i> sp. nov 4	<i>Neocosmospora yggdrasilica</i>	Yggdrasil, the World Tree, wide host range
<i>Neocosmospora</i> sp. nov 5	<i>Neocosmospora nidhoggi</i>	Níðhöggr, the serpent gnawing at Yggdrasil's roots, basal species to <i>N. yggdrasilica</i>
<i>Neocosmospora</i> sp. nov 6	<i>Neocosmospora lokii</i>	Loki, the trickster god, initially mistaken for another species
<i>Neocosmospora</i> sp. nov 7	<i>Neocosmospora jordiae</i>	Jord, the goddess of Earth and its creatures, isolated from a zoological garden





# Fungus - beetle association at greenhouse level

- > Novel species 1 only found in greenhouses 1 and 2
- > Sp. nov. 2 shared between greenhouses 1, 2 and 3
- > Greenhouse 3 has high fungal diversity with 5 novel species being found





# Biological relevance of novel fungal species

- › Stem trials
  - *Ficus, Acer, Betula, Fraxineus, Malus, Platanus, Populus, Quercus*
- › Living tree trials
  - *Acer, Betula, Malus, Platanus*
- › Of the novel species tested, 3 to 6 European tree species were (severely) affected



*Neocosmospora euwallaceae*



*Neocosmospora yggdrasillica*





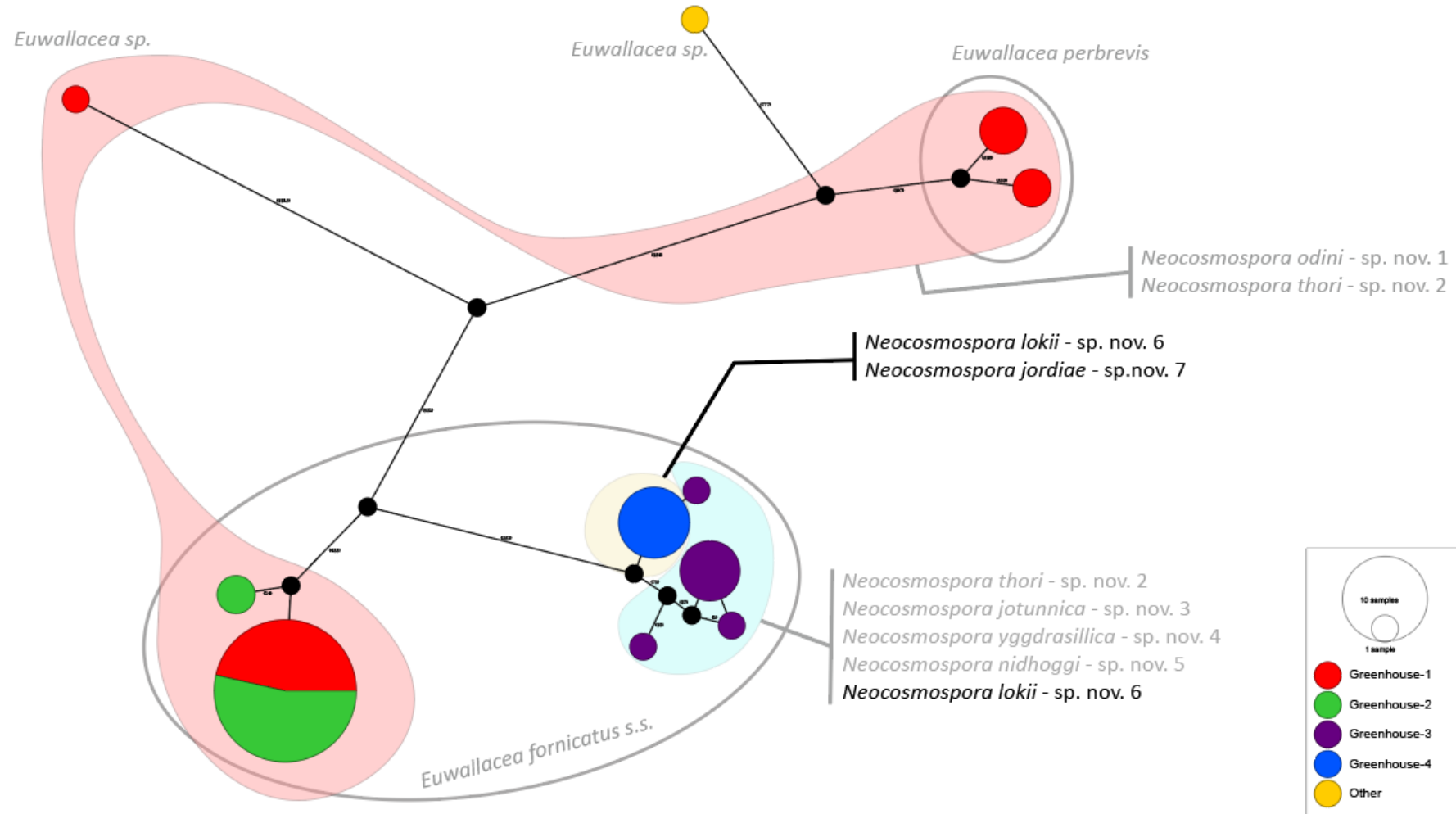
# *New outbreak*

- › Is the new outbreak linked to earlier outbreaks?
- › Use of both beetle and fungal species diversity





# Beetle-fungus combination suggests potential source or shared origin





# Conclusions

- › Outbreaks of regulated *E. fornicatus* s.s. were detected in several Dutch greenhouses
- › Novel *Neocosmospora* species were isolated from galleries and beetles
- › Analysis of complete beetle mtDNA in combination with its fungal community were instrumental in determining potential shared sources
- › An exclusive obligate symbiotic relation between *E. fornicatus* s.s. and *N. euwallaceae* is not true as the latter species was not detected at any of the outbreak sites, whereas other fungal species from the same clade were



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