

Molecular identification of *Hyalesthes*
and *Reptalus* species
(Hemiptera: Cixiidae) allows monitoring of
vector species throughout the year



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Hyalesthes and *Reptalus* spp: as vectors

Both *Hyalesthes* and *Reptalus* species are reported as vectors of **STOLBUR** phytoplasmas (*Candidatus* *Phytoplasma solani*, 16SrXII-A genetic group)

- *Hyalesthes obsoletus*: vector of stolbur diseases affecting several horticultural crops and grapevine
→ i.e. **Bois Noir (BN)**, a grapevine yellow (Maixner *et al.*, 1994).
- *Hyalesthes luteipes*: found positive to BN (Trivellone *et al.*, 2005).
- *Reptalus quinquecostatus*: can successfully inoculate BN into an artificial feeding medium through parafilm (Pinzauti *et al.*, 2008).
- *Reptalus panzeri*: found positive to BN (Palermo *et al.*, 2004), transmits the maize redness (Jović *et al.*, 2007).

Hyalesthes and *Reptalus*: species identification

Currently based on morphological features,
mainly concerning the **male genitalia**



The identification is restricted to specialist entomologists



These morphological features hamper the identification of juveniles and females



DNA-based approaches can help:
mitochondrial COI sequence
ITS2 ribosomal sequence

Hyalesthes and *Reptalus*: samples

✓ *Hyalesthes obsoletus*

✓ *Hyalesthes scotti*

✓ *Hyalesthes luteipes*



✓ *Reptalus cuspidatus*

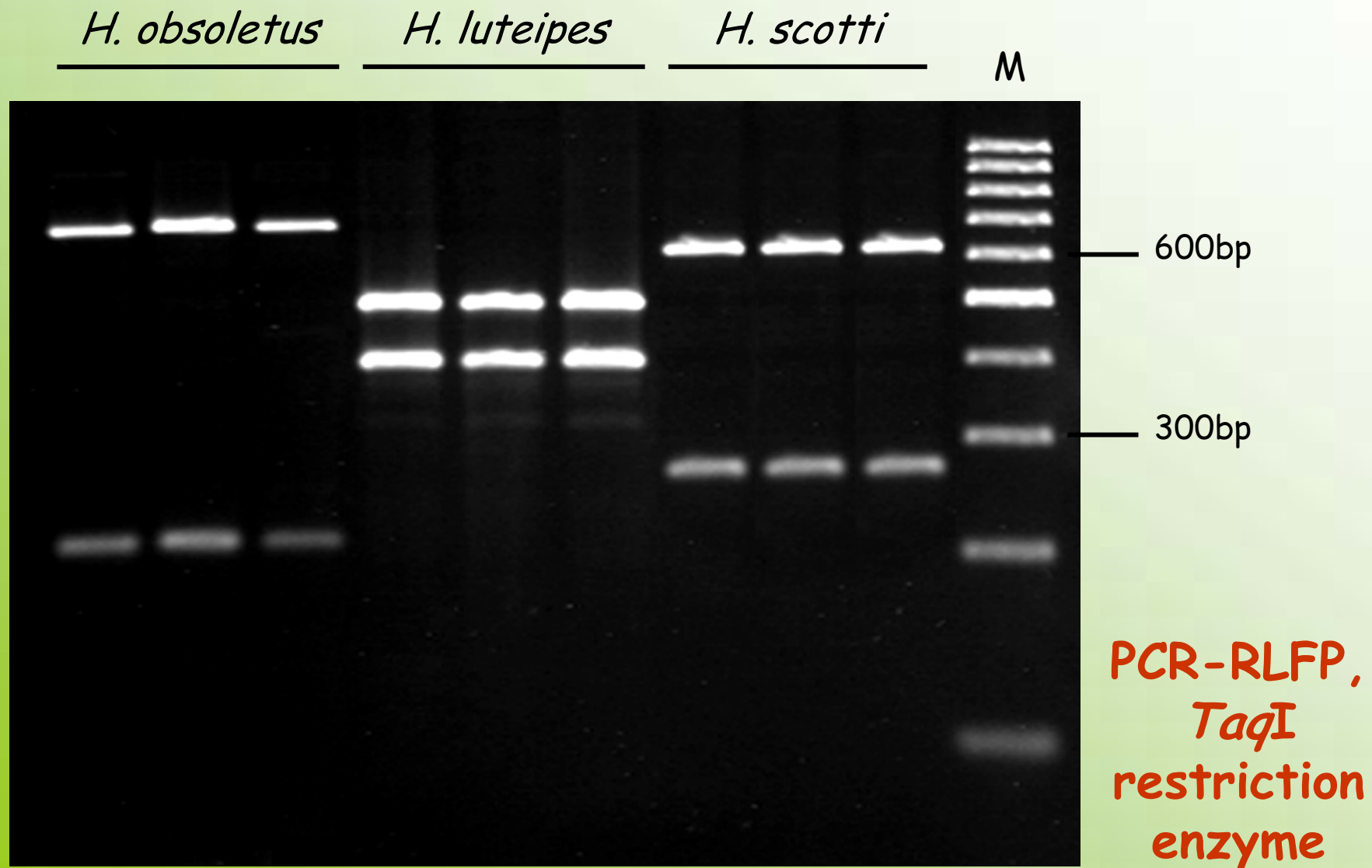
✓ *Reptalus quinquecostatus*

✓ *Reptalus panzeri*

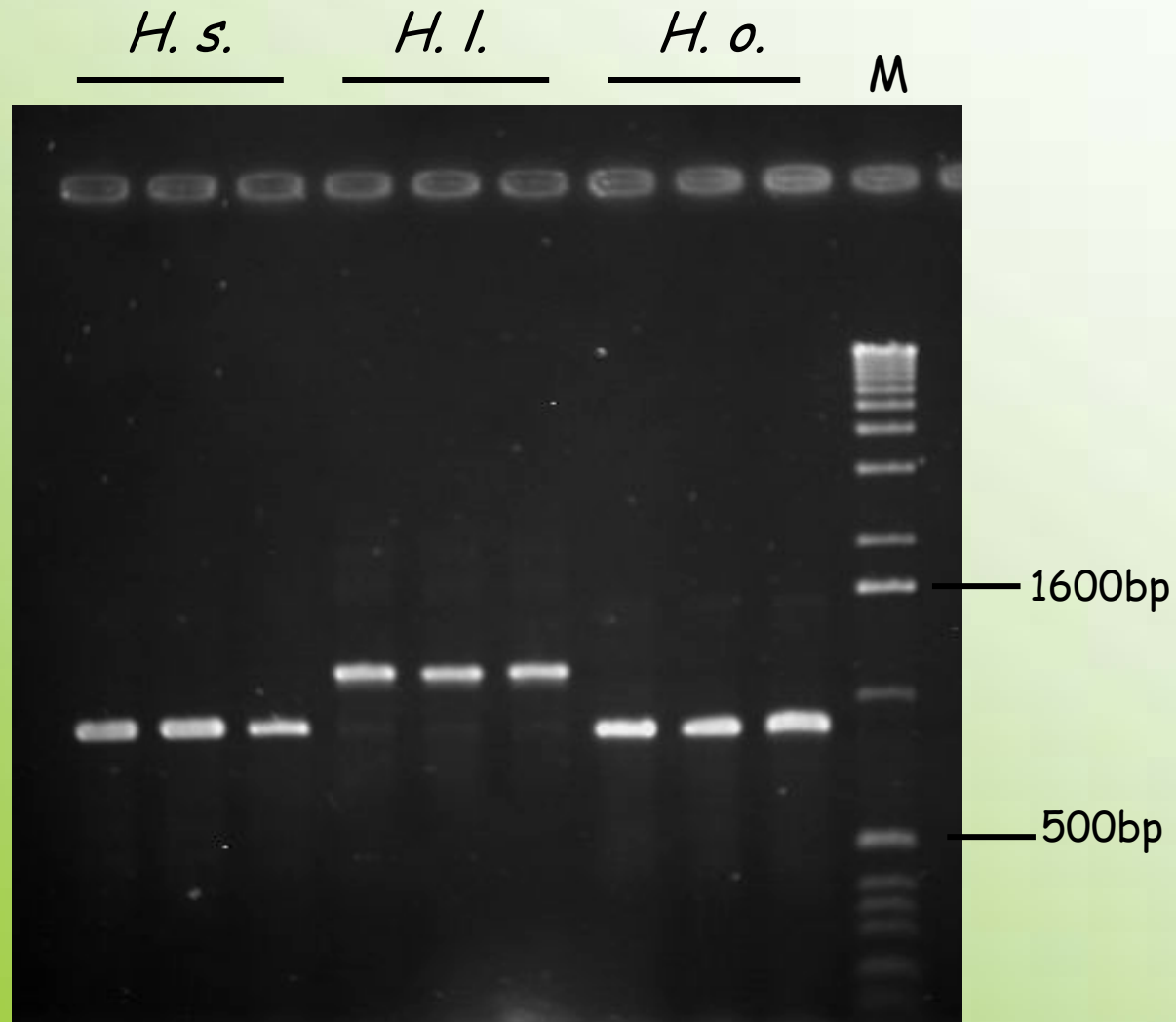
✓ *Reptalus melanochaetus*



Hyalesthes species - COI analysis

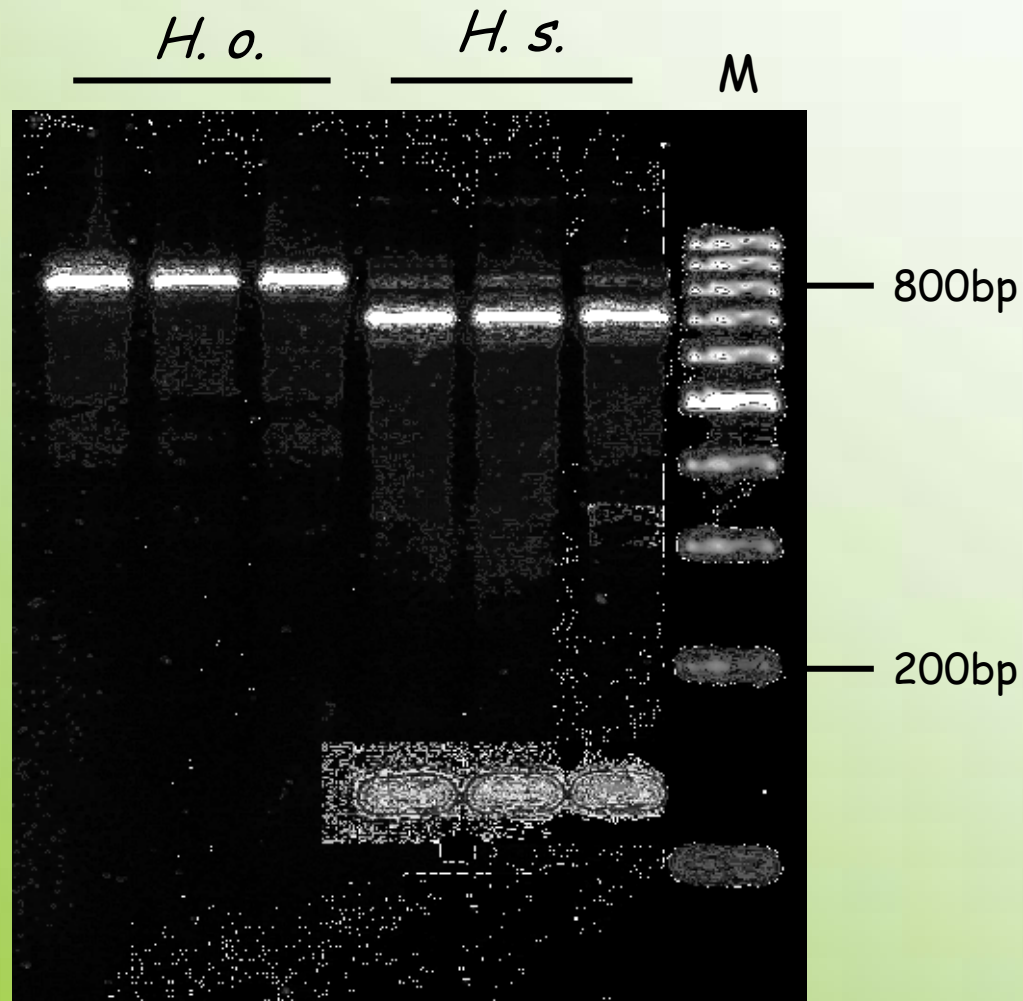


Hyalesthes species - ITS2 analysis



PCR assay

Hyalesthes species - ITS2 analysis



PCR-RLFP, *TaqI*
restriction enzyme

Reptalus species - COI analyses

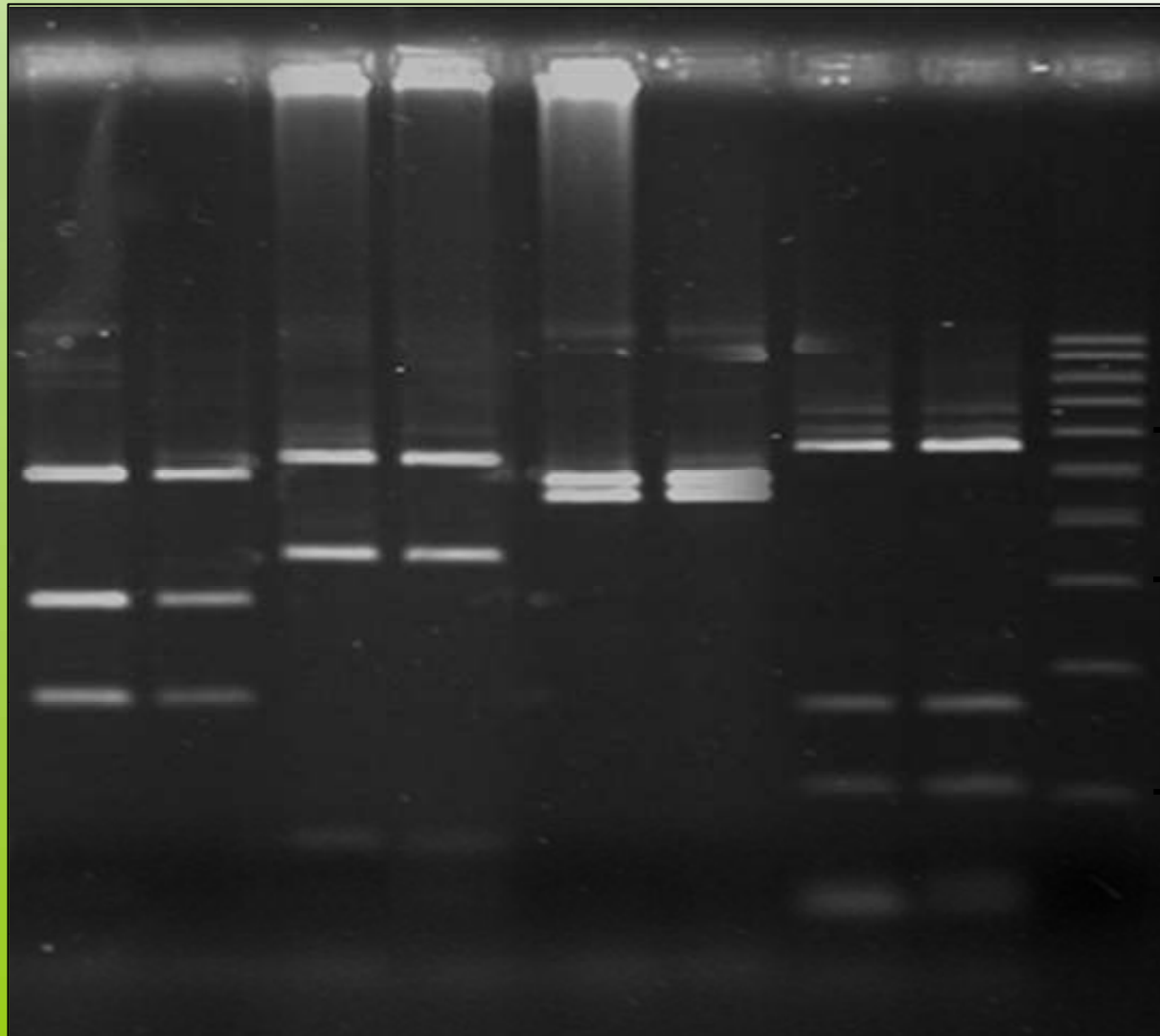
R. q.

R. c.

R. p.

R. m.

M



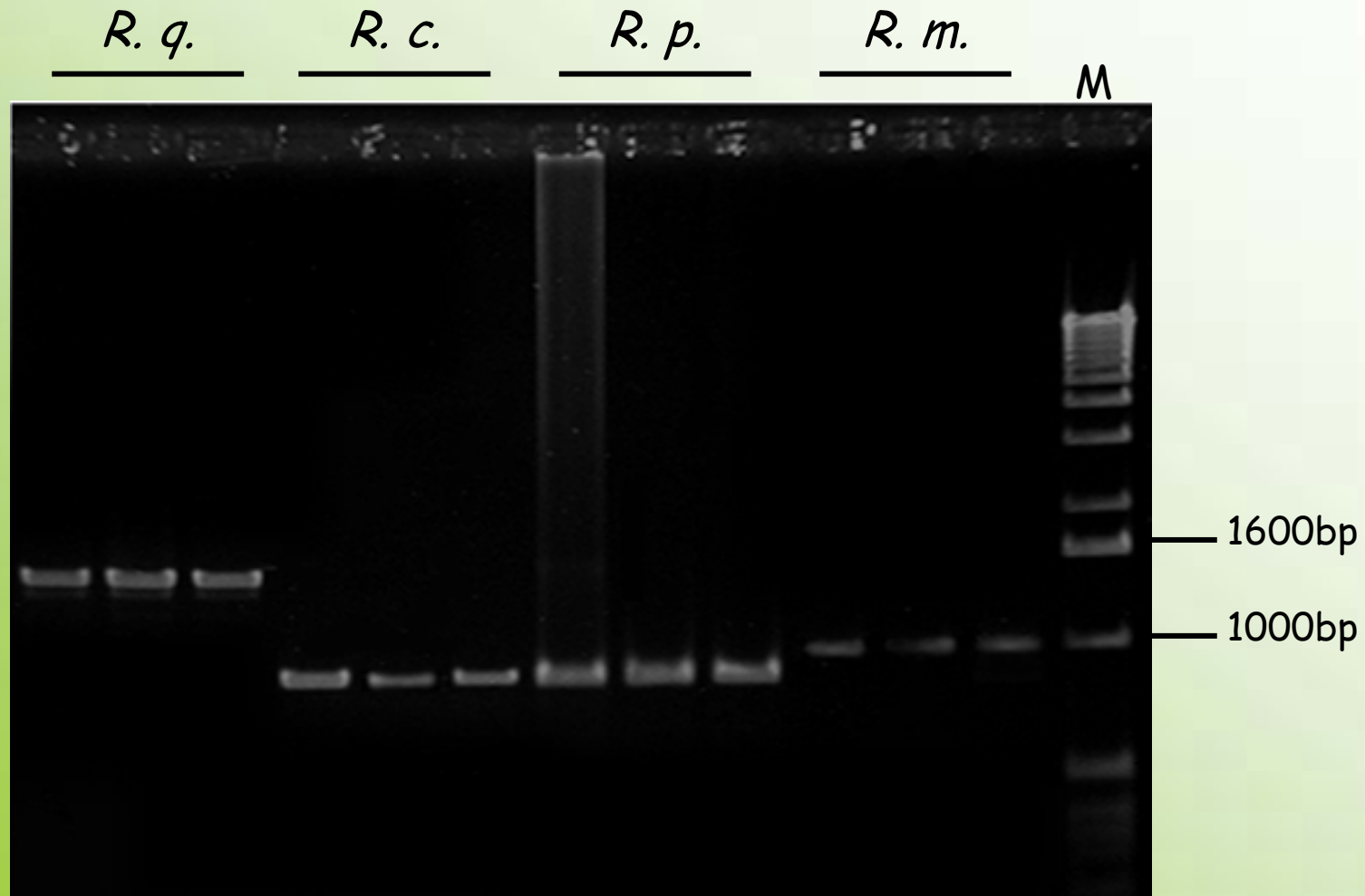
600bp

300bp

100bp

PCR-RLFP, *AluI*
restriction enzyme

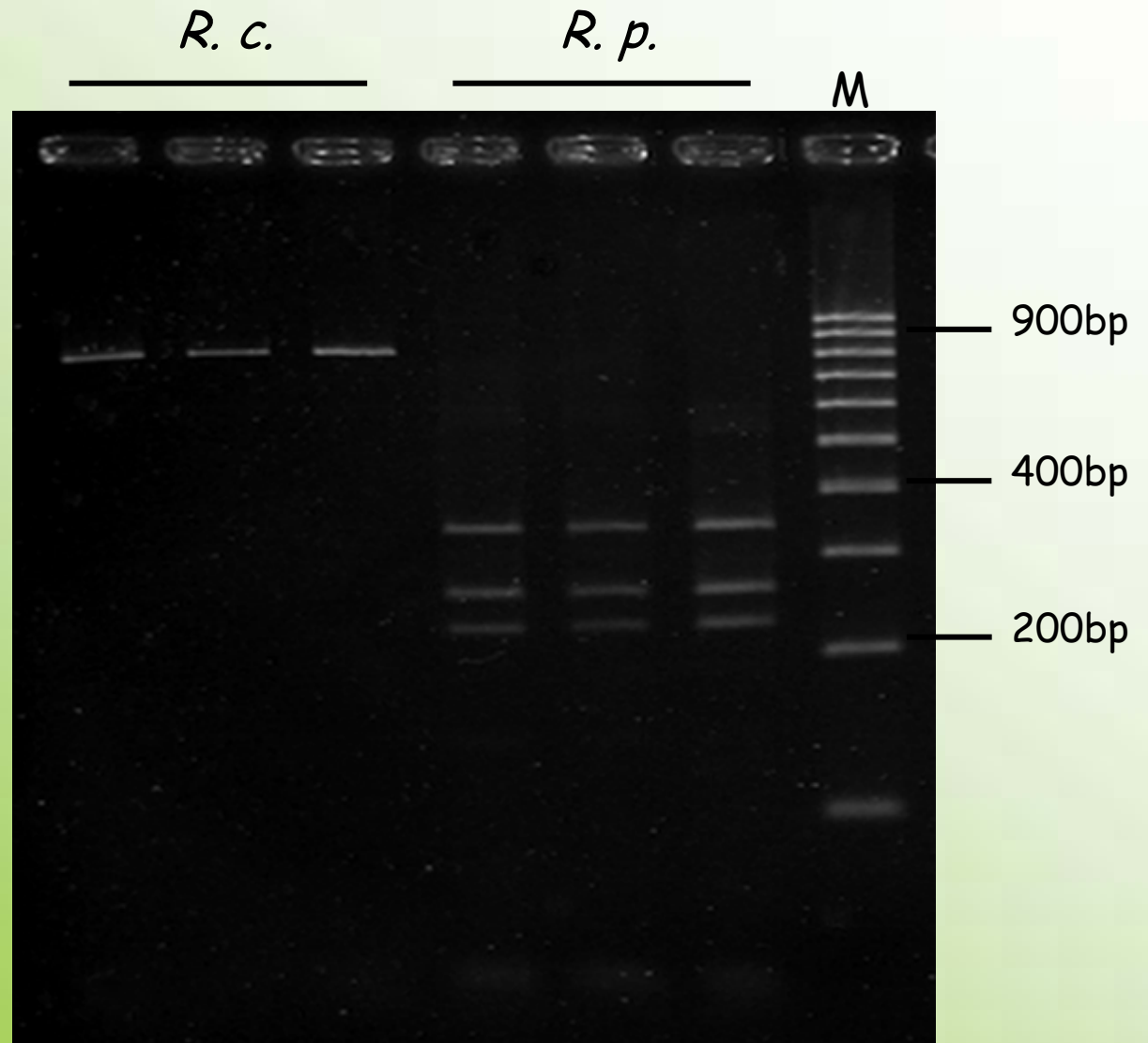
Reptalus species - ITS2 analyses



PCR assay

Reptalus species - ITS2 analyses

PCR-RLFP, *TaqI*
restriction enzyme



Discrimination among *Hyalesthes* and *Reptalus* species

- both COI and ITS2 markers are species-specific (no within-species polymorphisms have been recorded)
- no polymorphisms ascribable to different geographic origin and/or different host plants have been noticed within each considered species



Given their specificity and conservation both COI and ITS markers can be considered new reliable identification tools

New identification tools: advantages

- The new tools require rapid and handy procedures



They contribute to broaden the current handful of competent scientists

- The molecular assays can be applied to the species identification of juveniles and females



They contribute to faunal studies and insect vectors monitoring:

- i) extending the collection period to the whole year
- ii) associating cixiids with their actual host plants



Thanks for your attention!

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