Biosecurity measures for *Bombus* spp.

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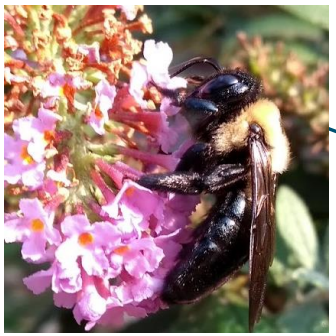
Istituto Zooprofilattico Sperimentale
delle Regioni Lazio e Toscana

OIE

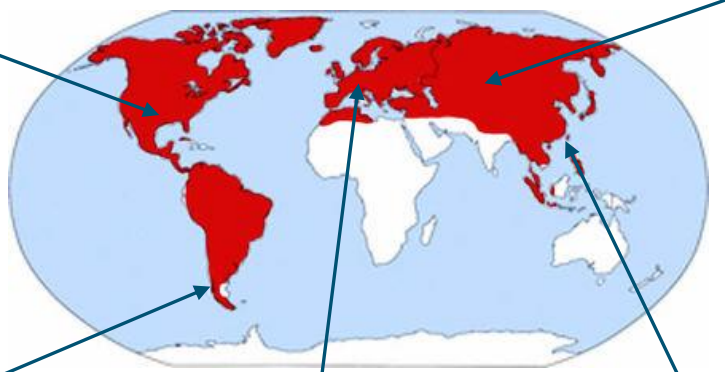
Bombus spp.

- Bumblebees: Genus ***Bombus***
- ≈ 250 species worldwide
- ≈45 species of Cuckoo bumblebees (subgenus *Psithyrus*)
- 'true' *Bombus:* primitively eusocial (annual cycle)



Bombus griseocollis. Photo: D. Panziera

The giant Patagonian bumblebee, *Bombus dahlbomii*. Photo : C.L. Morales



Bombus morawitzi Credit: P. Rasmont

Bombus brodmannicus male. Credit: P. Rasmont

Bombus formosellus

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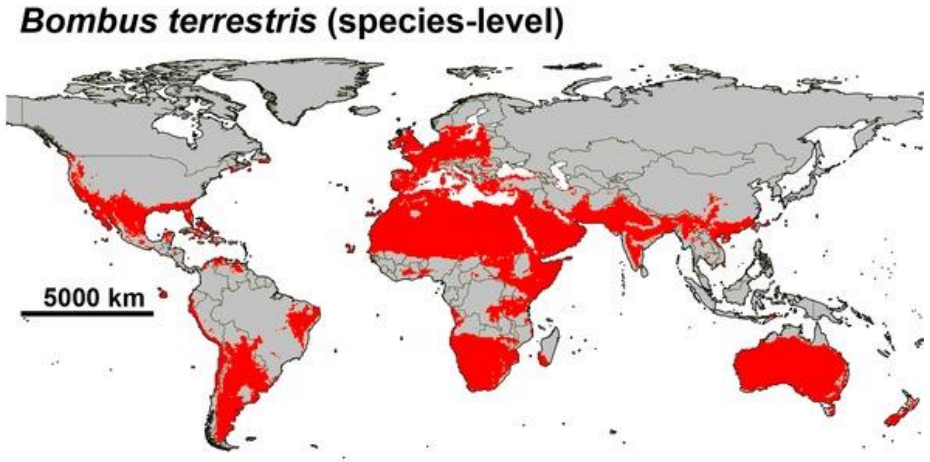
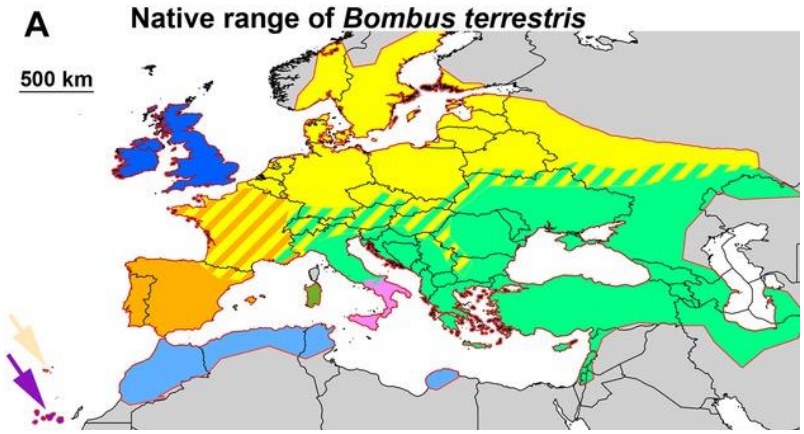
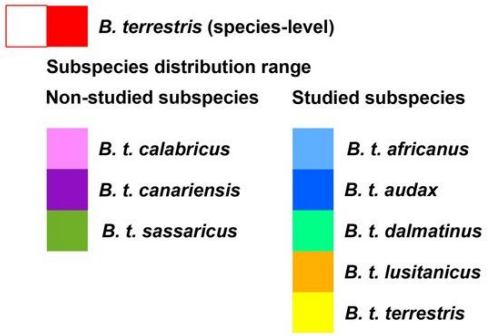
Bombus spp. as managed pollinators







From Lecocq *et al.*, 2016

Bombus spp. as managed pollinators

- **1985**: Bumblebees more effective than hand pollination for greenhouse tomatoes (hand pollination=10 keuro/ha/year)
- **1987**: start of commercial rearing (Biobest, (BE))
- **1988**: Koppert (NL) starts bumblebee rearing
- 5 species reared: ***B.terrestris*** *(EU),* ***B.impatiens*** and *B.occidentalis* (USA), *B.ignites* (Japan) and *B. lucorum* (East Asia)

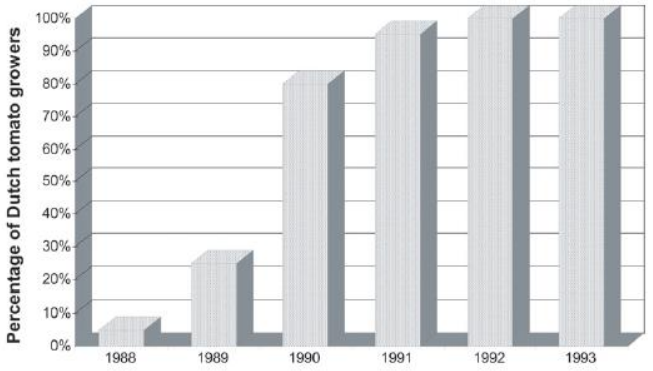


Figure 1. Application of bumblebees for pollination by Dutch tomato growers since the start of commercial rearing in 1987.
Velthuis and van Doorn, 2006

Bombus terrestris Photo: D. Panziera

Bombus impatiens

Bombus impatiens Photo: Koppert

Bombus terrestris

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Commercial rearing of *Bombus* spp.

- Annual production: **1 million** colonies (2004)

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Animal health law (EU) 2016/429

Adopted by EU commission in 2016: Regulation (EU) on transmissible animal diseases ("Animal Health Law") applicable since **21 April 2021.**

entry is permitted only for colonies:

- bred, reared and packaged under **environmentally controlled conditions**
- in establishments and which can be checked to be free of the **small hive beetle.**

European Commission

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Animal health law (EU) 2016/429

Article 64: Dispatch to the Union of honeybees and bumble bees

- Consignments of queens of honeybees and bumble bees shall only be permitted to enter the Union if they comply with the following requirements:

(a)the packaging material and queen cages used to dispatch the honeybees and bumble bees into the Union must:

(i)be new;

(ii)not have been in contact with any bees and brood combs;

(iii)have been subject to all precautions to prevent their contamination with pathogens causing diseases [...]

(b)the feed products [...] must be free from pathogens causing their diseases;

(c)the packaging material and accompanying products **must have undergone a visual examination before dispatch** to the Union to ensure that they do not pose an animal health risk and do not contain:

[...]

(ii)in the case of bumble bees, **small hive beetle**, in any of their life stages.

Article 69 : The establishment of origin of bumble bees

Consignments of bumble bees shall only be permitted to enter the Union if they:

(a)have been bred and kept in an environmentally isolated bumblebee production establishment which:

(i)has facilities which ensure that the production of bumble bees is carried out inside of a flying insect-proof building;

(ii)has facilities and equipment which ensure that the bumble bees are further isolated in separate epidemiological units and each colony in closed containers within the building throughout the whole production;

(iii)the storage and handling of pollen [...] is isolated from the bumble bees throughout the whole production of bumble bees until it is fed to them;

(iii)has standard operating procedures to prevent [...] and to regularly survey for the presence of **small hive beetle** within the establishment;

(b)within the establishment referred to in point (a), the bumble bees must come from an epidemiological unit in which small hive beetle has not been detected.

- **Article 70 : The consignment of bumble bees**

Consignments of bumble bees shall only be permitted to enter the Union if they have been dispatched to the Union in **closed containers**, each containing a colony of a **maximum of 200 adult bumble bees**, with or without a queen.

- **Article 71 : Handling after the entry of honeybees and bumble bees**

[...] 3.Operators receiving bumble bees shall destroy their container and the packaging material that accompanied them from the third country or territory of origin but they may keep them in the container in which they entered into the Union until the end of the lifespan of the colony.

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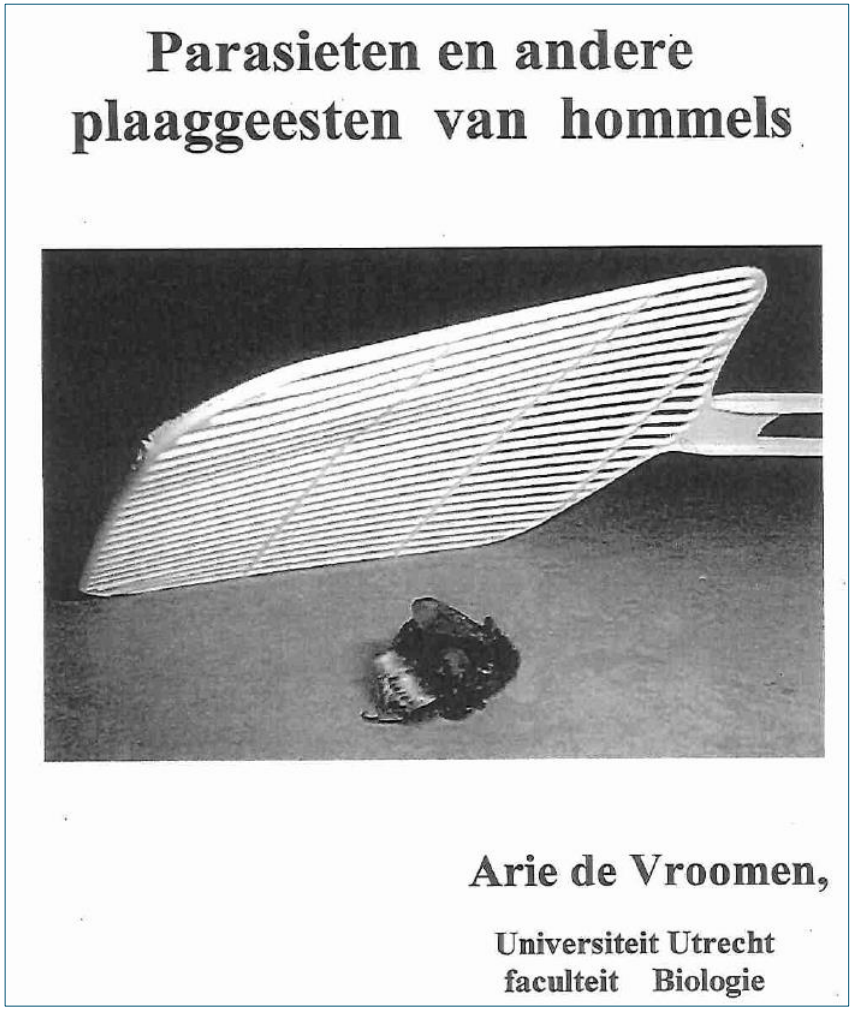
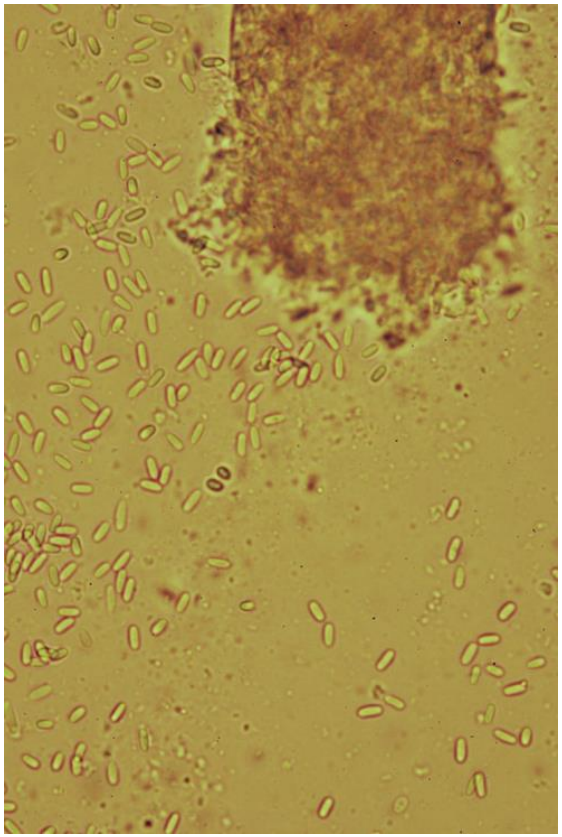
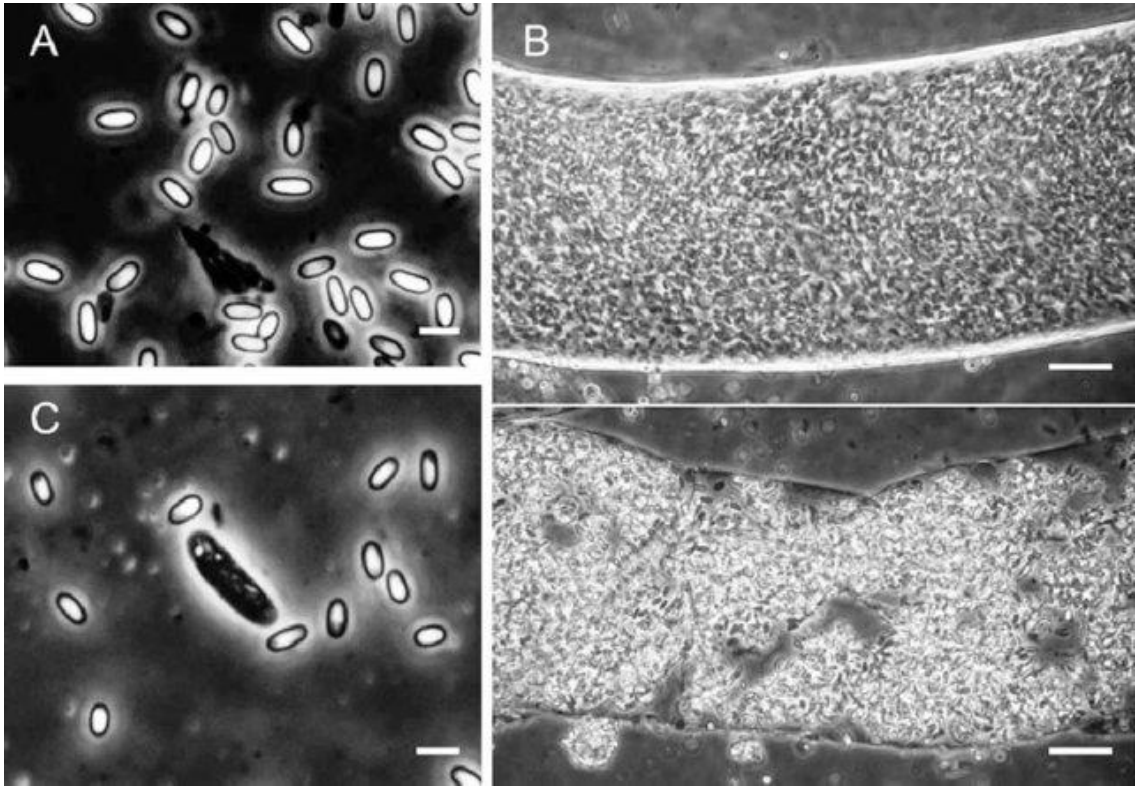
Parasites of Bumblebees



Internal parasites of *Bombus* spp.: Microsporidia



Nosema bombi



Nosema bombi
A: Spores.
B: Parts of Malpighian tubules of B. terrestris heavily filled with spores packed together tightly (upper) and more loosely (lower).
C: Immature oocyst of the neogregarine Apicystis bombi among spores of N. bombi from the only mixed infection found. [Bars: A-C: 5 mm; B: 25 mm; Phase-Contrast Microscopy].

From Plischuk *et al.,* 2017

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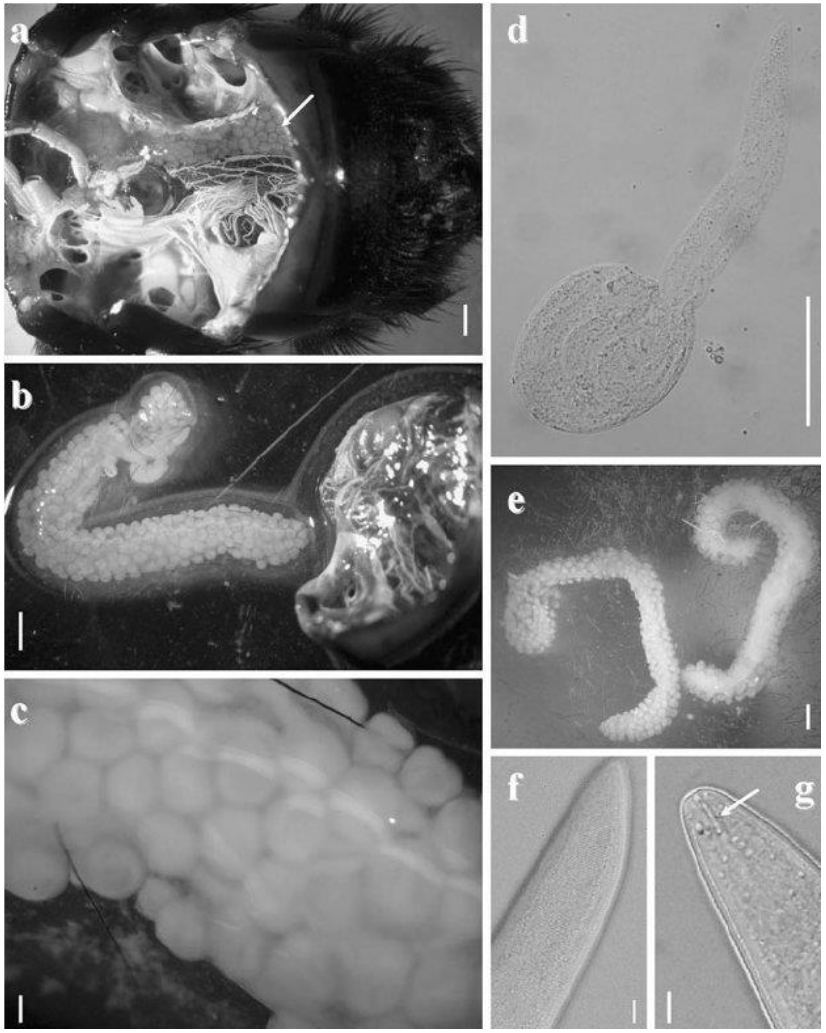
Internal parasites of *Bombus* spp.: Nematodes

Sphaerularia bombi:

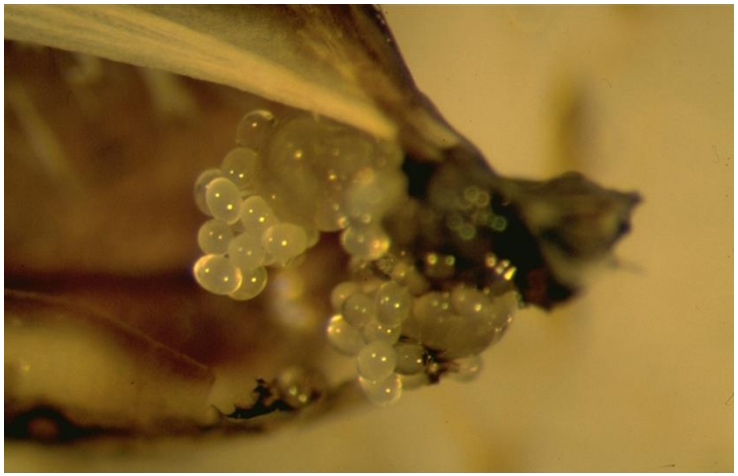
a Dissected bumble bee showing sac in the hemocoel (arrow) (bar: 1 mm).
b Ovarian sac (bar: 1 mm).
c Closer view of part of an ovarian sac (bar: 100 µ m).
d Juvenile emerging from an egg (bar: 100 µ m).
e Two ovarian sacs, numerous juveniles and eggs (bar: 1 mm).
f Anterior end of a juvenile showing annulated cuticle (bar: 10 µ m).
g Anterior end of a juvenile showing stylet (arrow) (bar: 10 µ m)

(From Plischuk and Lange, 2012)

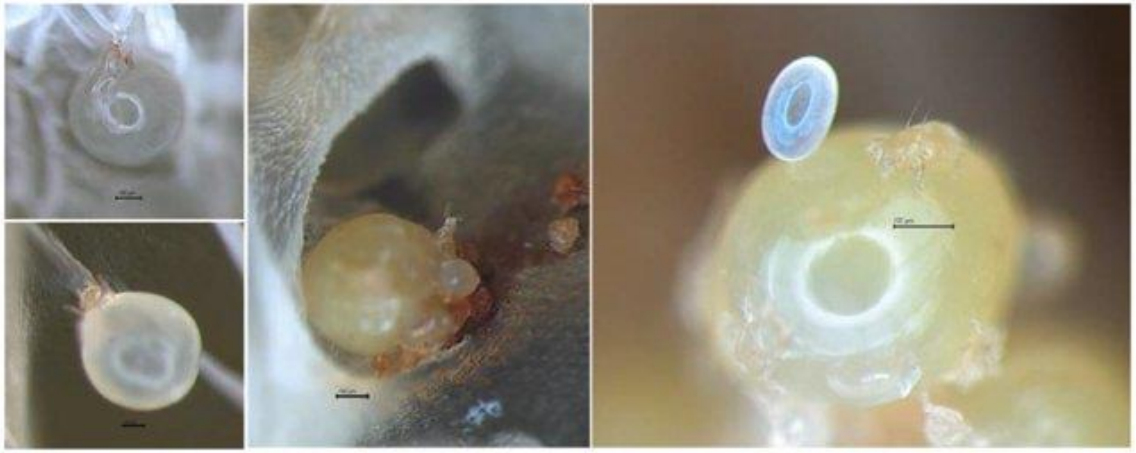


Sphaerularia bombi (nematode), Hypertrophied ovary:

Internal parasites of *Bombus* spp.: mites



Locustacarus buchneri (Tracheal mite)



Adult female of *Locustacarus buchneri* in air sac of *Bombus terrestris*

From Keum *et al.,* 2021

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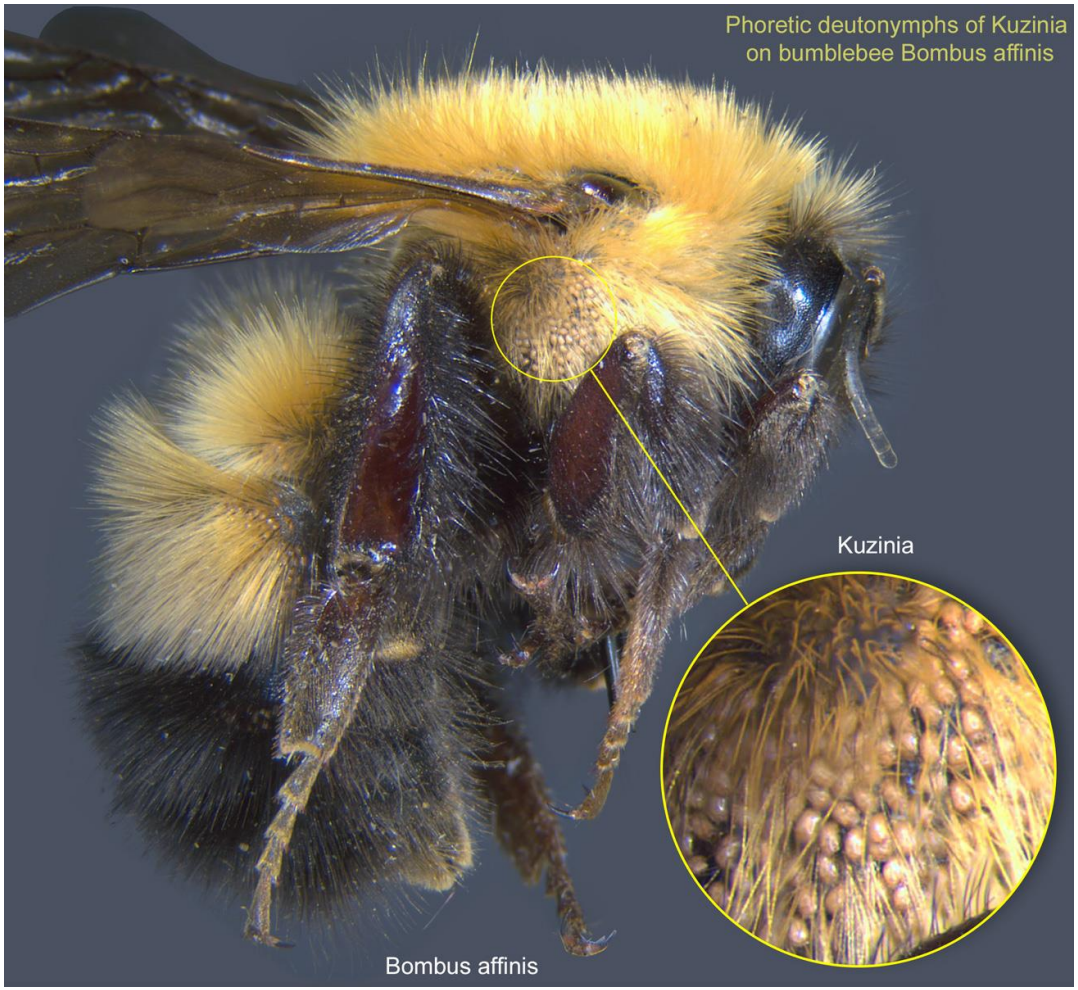
External parasites: Mites (1)

- *Kuzinia laevis*
  - Small mite species: 0.25mm length



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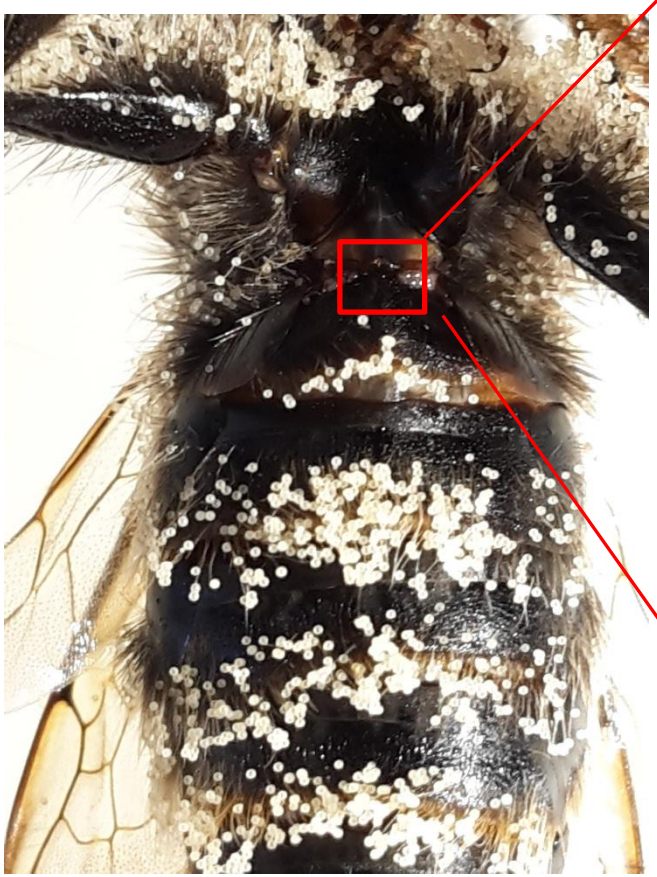
Kuzinia laevis



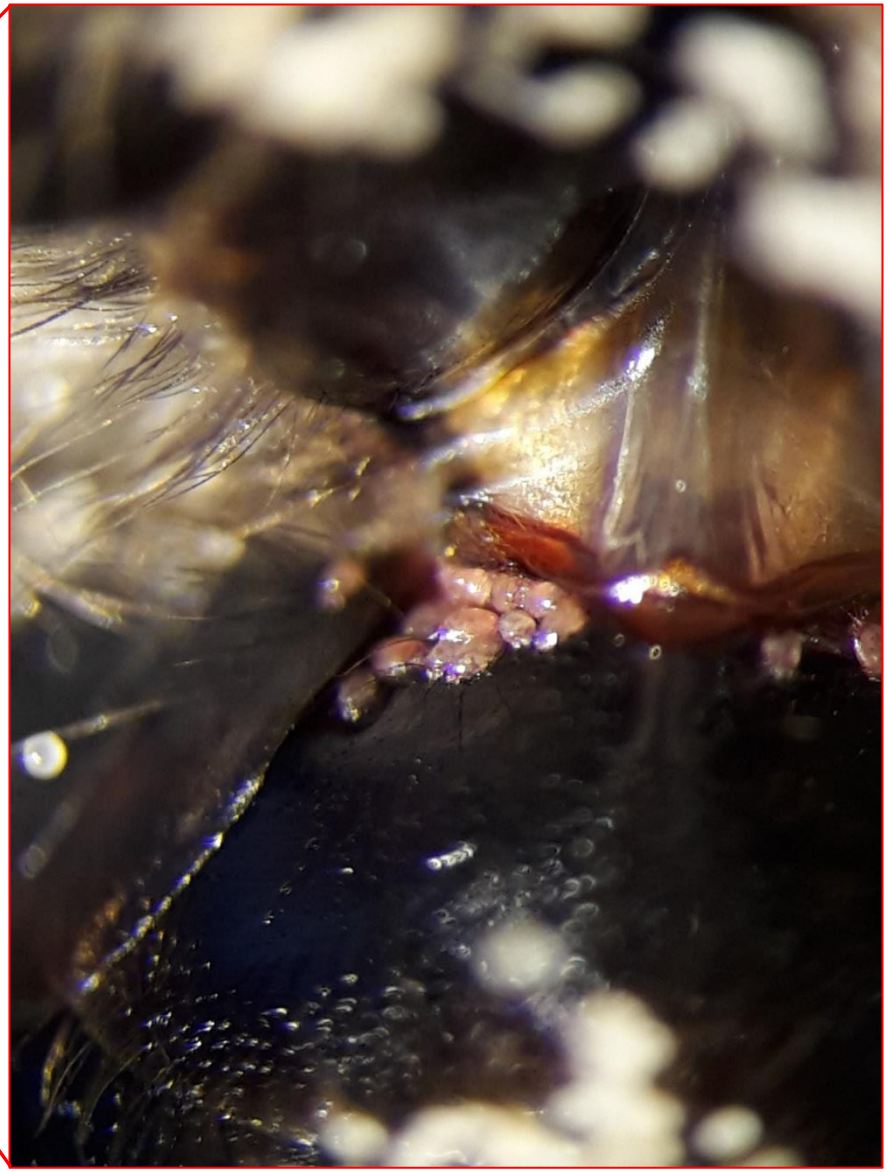


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Photo: Bram Cornelissen

Parasitic wasp *Melittobia*

Melittobia acasta

- 2mm long
- Females: black with brown/yellow legs
- Males: brown with recognisable antennae
- Specific to *Bombus* spp.

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Parasitic wasp *Melittobia*

Developmental stages of *Melittobia acasta* (Walker, 1839):

A: an egg

B–C: mature larvae

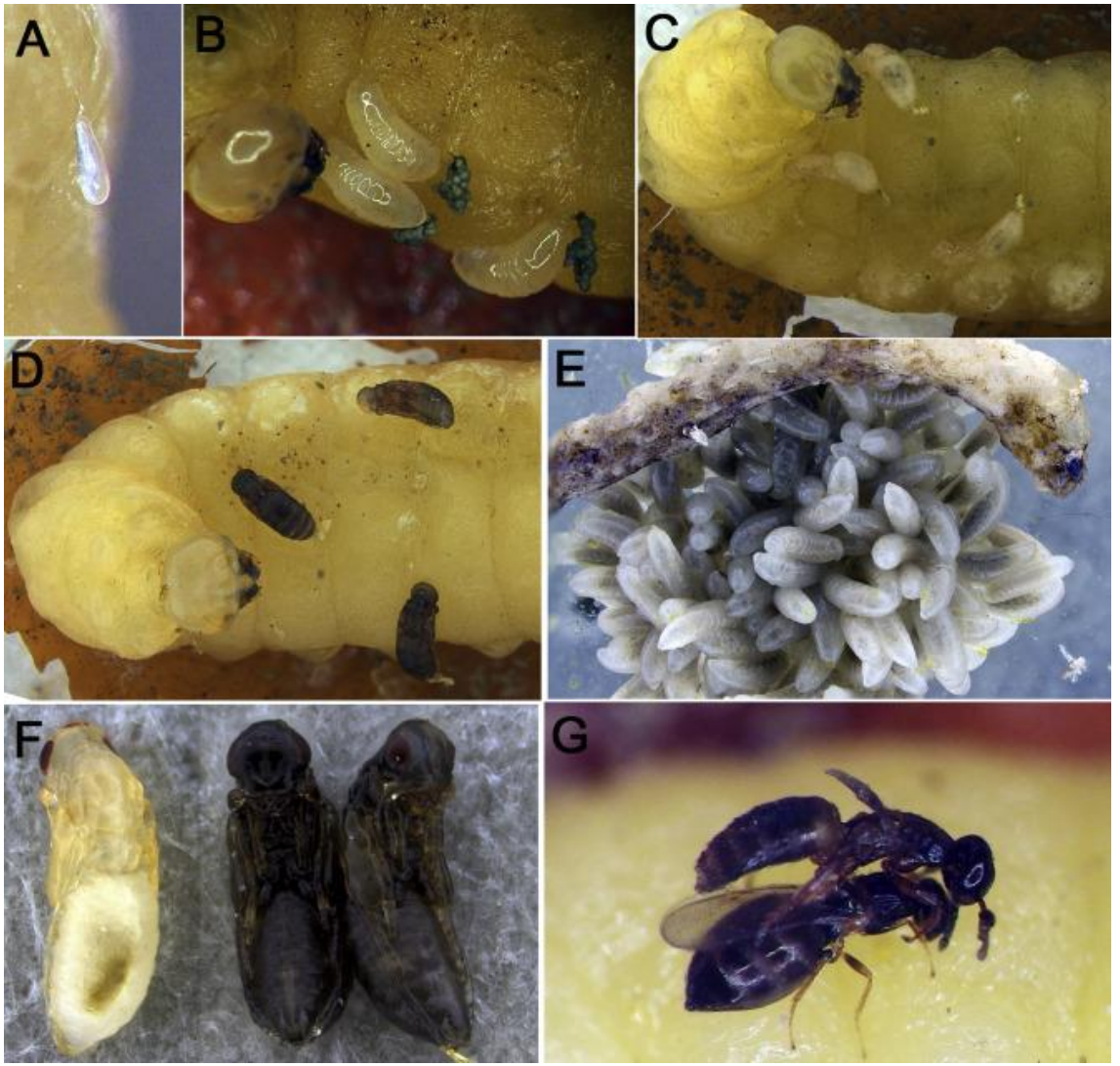
D: pupae

E: gregarious larvae on a host larva;

F: female pupae of the early stage (left) and later stage (right)

G: courtship behavior of male (on top) and female (on bottom).

From Lee and Kim, 2019



Notifiable Honeybee parasites : Mites (2)

Tropilaelaps mites

- 1mm long

Photographs: Patcharin Phokasem, Chiang Mai University (Thailand)

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Notifiable Honeybee parasites : Mites (3)

Varroa

- 1,5mm

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Notifiable Honeybee parasites : Small hive beetle

- Adult: 5-7mm
- Larvae:11mm long
- in South Italy since 2014

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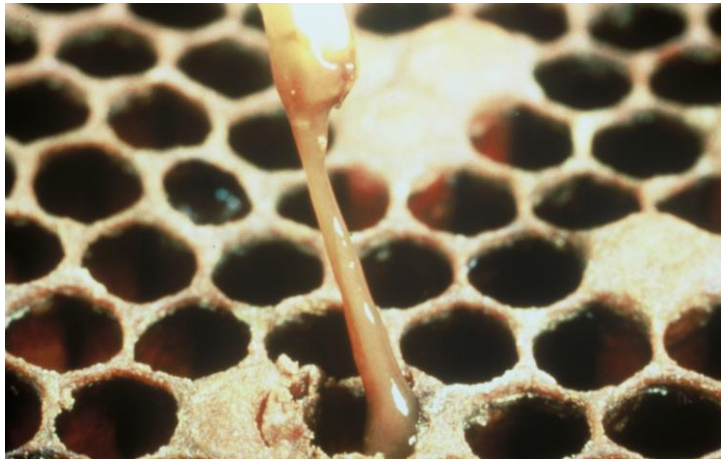
Notifiable Honeybee parasites : Brood diseases

American Foulbrood

- Bacteria
- Brood disease



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Other parasites of concern

Table 1. Parasites and pathogens of bumble bees that may be transmitted from a reservoir population to sympatric wild populations (i.e., spillover).[a]

Parasite	*Taxon*	*Host genus*	*Generalist*[b]	*Commercial*[c]	*Transmission*[d]	*Virulence*	*Spillover*[e]
Aethina tumida	Arthropoda	*Apis*	yes?	yes	?	?	no
Apicys tisbombi	Apicomplexa	*Bombus*	yes	yes	?	high?	no
Crithidia bombi	Euglenozoa	*Bombus*	yes	yes?	high	high	yes
Crithidia expoeki	Euglenozoa	*Bombus*	yes	?	high?	high?	yes?
Locustacarus buchneri	Arthropoda	*Bombus*	yes	yes/no[f]	?	?	yes?
Nosema bombi	Microsporidia	*Bombus*	yes	yes	medium	high	no
Nosema ceranae	Microsporidia	*Apis*?	?	?	?	?	no
Acute bee paralysis virus	Group IV (+)ssRNA[g] virus	*Apis?*	?	?	?	?	?
Black queen cell virus	Group IV (+)ssRNA virus	*Apis?*	?	yes	?	?	?
Deformed wing virus	Group IV (+)ssRNA virus	*Apis?*	?	yes	?	high	?
Israeli acute paralysis virus	Group IV (+)ssRNA virus	*Apis?*	?	yes	?	?	?
Kashmir bee virus	Group IV (+)ssRNA virus	*Apis?*	?	yes	?	?	?
Sac Brood virus	Group IV (+)ssRNA virus	*Apis?*	?	?	?	?	?

[a] *Question mark indicates the absence of evidence or doubt about status.*
[b] *Multihost parasite.*
[c] *Present in commercial colonies.*
[d] *Intercolony transmission.*
[e] *Evidence for pathogen spillover.*
[f] *Present in commercial colonies, but now appears to be controlled.*
[g] *Positive-sense single-stranded RNA viruses.*

Meeus, I., Brown, M. J., De Graaf, D. C., & Smagghe, G. U. Y. (2011). Effects of invasive parasites on bumble bee declines. *Conservation Biology*, *25*(4), 662-671.

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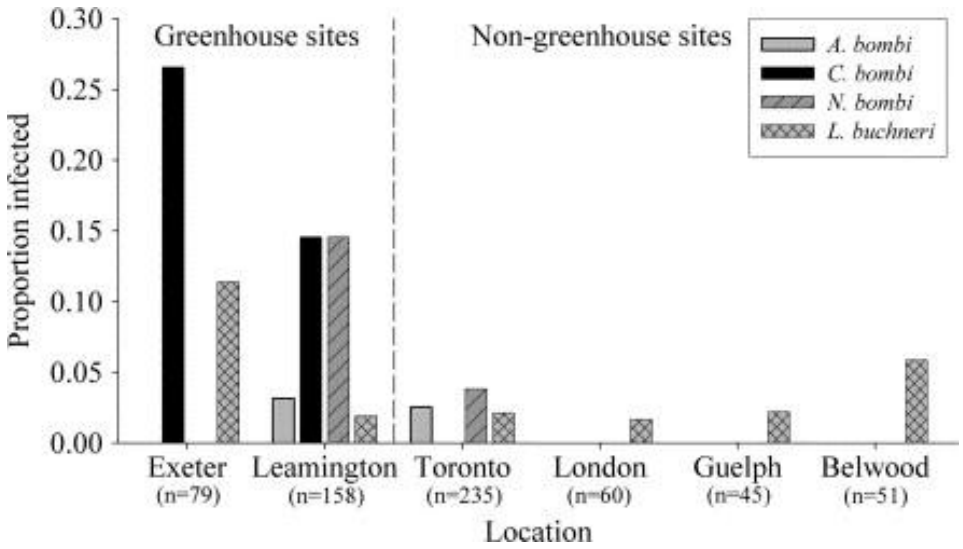
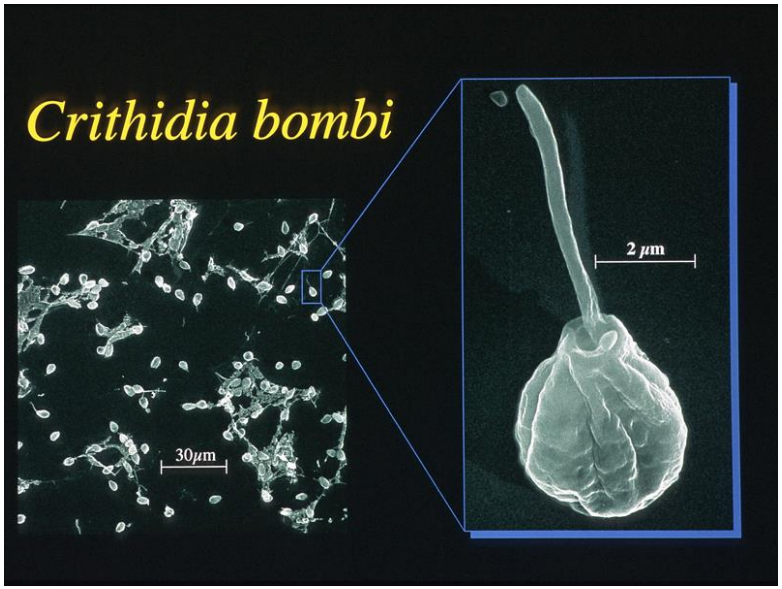
Other parasites: Chritidia



Proportion of bumble bees (all species pooled) infected by four parasites,

From Colla *et al*., 2006



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Summary

- 2 main species used worldwide for pollination: *B.terrestris* and *B.impatiens*
- More than 1 million colonies traded each year
- Several specialised parasites but may harbour honeybee parasites
- Production facilities and random samples are checked

Perspectives

- Are not present in the monitored parasites:

-viruses

-*Chritidia bombi*

=> Potential spillover

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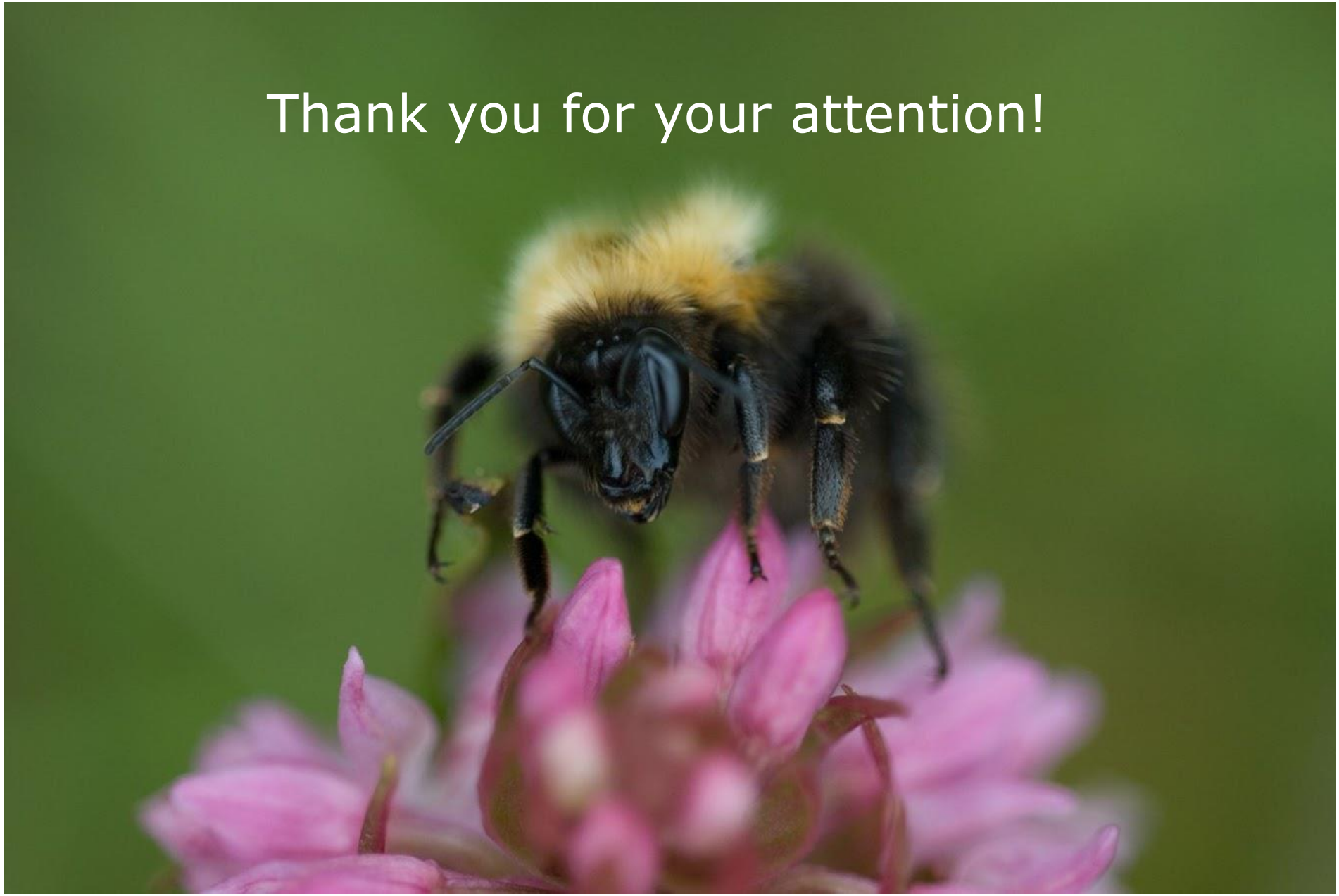
Thank you for your attention!



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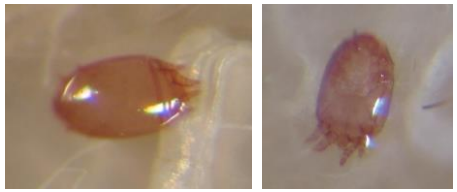
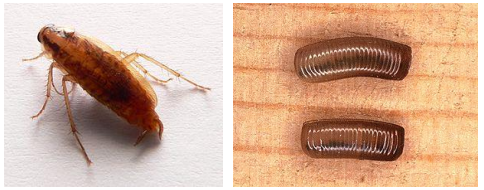
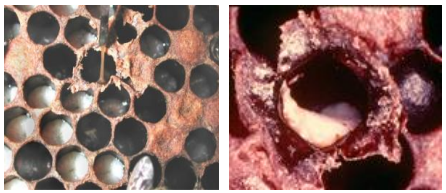
Bee pests and diseases

Type	Visual recognition	Characteristics
Mite (*Varroa destructor*)		• Tiny mite (1.1 mm in width and 1.5 mm in length) • Eight legs • Reddish brown • Only on honeybees
Mite (*Tropilaelaps spp.)*		• Tiny mite (1 mm in length) • Eight legs • Reddish brown • Fast running mite • Only on honeybees
Mite (*Kuzinia laevis*)		• Very tiny mite (0.250 mm in length), and therefore difficult to see • Eight legs • Brownish • Only on bumblebees
Small hive beetle (*Aethina tumida)*		• Tiny beetle (5 – 7 mm) • Both beetle and larvae 6 legs • Beetle brown, larvae beige (10 - 11 mm long, 3 pair of legs, stings on the dorsal side, deposits a greasy trace) • Honeybee and bumblebee pest
Wasp *(Melittobia acasta)*		• Tiny wasp (max. 2 mm in length) • Female: black wasp with brown-yellowish legs and antennae base (pic. left) • Male: brownish, smaller wings and striking antennae (pic. right) • Only on bumblebees
Wasp *(Bracon hebetor)*		• Tiny wasp (+/- 1 to 3 mm in length) • Black wasp with brownish legs and black wings except for the last one third of the wings (= transparent) • Does not feed on bees (only on other insects)

Type	Visual recognition	Characteristics
Cockroach (*Blattella germanica*)		• Small cockroach (+/- 10 mm in length) • Brown with long antennae • Eggs brown, long, knurled (pic. right) • Feeds on debris
Cockroach (*Periplaneta americana*)		• Cockroach (+/- 45 mm in length) • Brown with long antennae • Nymphs smaller without wings (pic. right) • Feeds on debris
Moth *(Vitula edmandsii)*		• Small moth (+/- 10 mm in length) • Blue-greyish with black markings on wings • Hind wings light grey • Feeds on debris
Moth (*Plodia interpunc-tella*)		• Small moth (8-10 mm in length) • White-greyish band on wings, tip of wings red-brownish • Hind wings light grey • Feeds on debris • Adult larvae 12 mm length
Clinical signs of European or American foulbrood disease in honey bee brood		• Disease caused by bacteria (*Melissococcus plutonius* EFB or *Paenibacillus larvae AFB)* • Bee larvae die young • Dead bee larvae are sticky and smelly, and are 'sunken' in their cells (AFB), only in honeybee brood
Cliniocal signs of chalkbrood in honey bee brood.		• Disease caused by fungi (*Ascosphaera apis chalcbrood figure* or *Aspergillus sp stonebrood no figure)* • Larvae become covered with fungi and then harden like pieces of chalk or stone • Only in honeybee brood