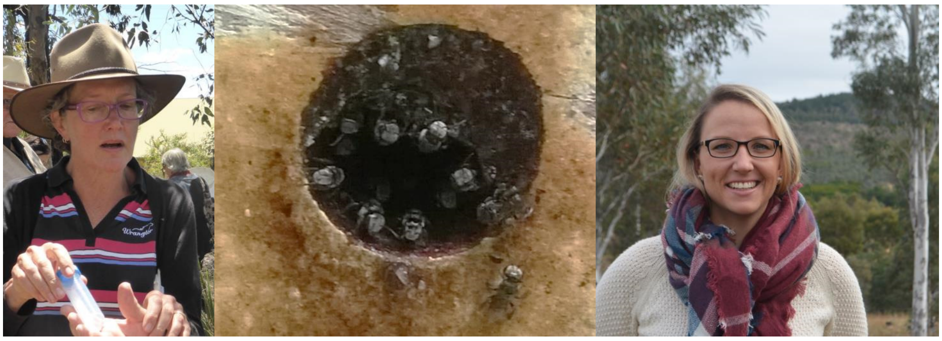
STINGLESS BEE PESTS AND DISEASES



ROBERT SPOONER-HART, MEGAN HALCROFT AND JENNY SHANKS

WESTERN SYDNEY UNIVERSITY

WITHIN COLONY PARASITES OF STINGLESS BEES IN AUSTRALIA

- native hive syrphid fly
- native hive phorid fly
- introduced small hive beetle
- native hive/pollen beetles
- mites
- soldier fly

HIVE SYRPHID FLY *Ceriana ornata*

- Common in stingless hives being manipulated, esp. during splitting for propagation. Adults attracted to hive stores
- Will also enter weak or dead hives
- Lay eggs on hive structures, or in hive external cracks/joins after hive splitting, honey removal

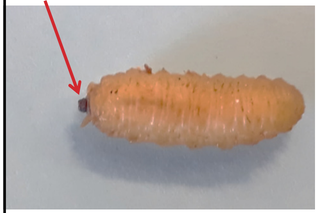
spiracles

Pre-pupal stage

- Larvae hatch from eggs burrow through gaps in propolis
- Maggot-like, 2 posterior spiracles
- Feed on hive stores, gradually destroying hives, slime out in severe cases
- Pre-pupal larvae leave hive to pupate in soil , or pupate in hive

Larvae in stingless bee hive structures

spiracles

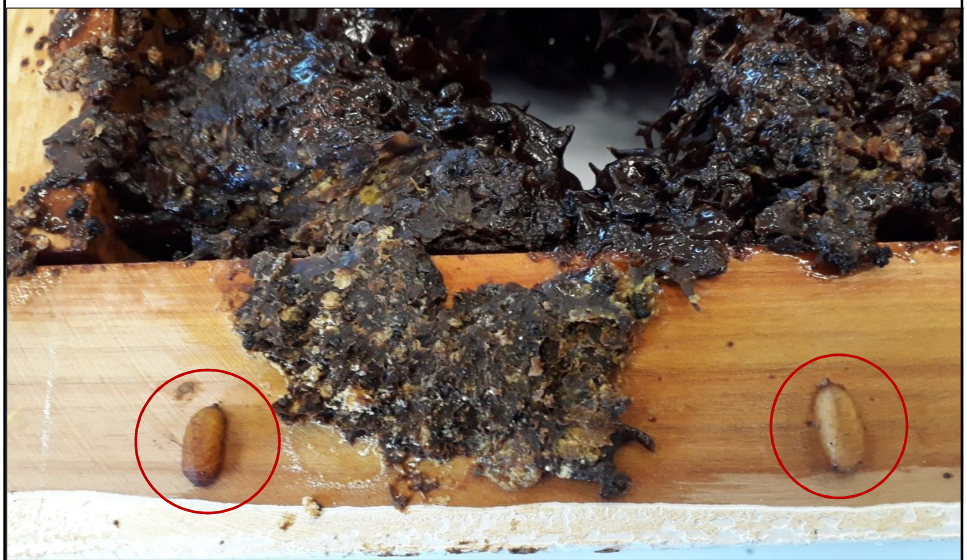


Pre-pupal stage

- Larvae hatch from eggs burrow through gaps in propolis
- Maggot-like, 2 large posterior spiracles
- Feed on hive stores, gradually destroying hives, slime out in severe cases
- Pre-pupal larvae leave hive to pupate in soil , or pupate in hive

Larvae in stingless bee hive structures

Syrphids pupating in stingless bee box



Management: Protect against infestation by taping up hive cracks/seams after splitting or opening

HIVE PHORID FLY *Dohrniphora trigoniae*

- Hump-backed small flies, run rather than fly when disturbed
- Enter hives through the entrance esp. if weak hives, or during hive manipulation
- Larvae small, maggot-like, feed on stores. Pupate in hive
- Protect hives by reducing size of hive entrances, and using traps

A. Phorids outside hive entrance

B. Phorid (and small ant!) trap

SMALL HIVE BEETLE *Aethina tumida*

- Will infest stingless bee hives, but less frequently infested than *Apis mellifera*. However, unlike *A. mellifera*, most stingless bees are kept under climatic conditions suitable for SHB populations
- Mainly enter weak or dead stingless bee hives
- Adult beetles enter usually around dusk
- Lay eggs in crevices and corners

- Larvae have distinct head and 3 pairs of thoracic legs
- Consume stores, cause fermentation and “slime outs”
- Larvae leave hive to pupate in soil

- SHB management similar for honeybees. Reduce entrance size, beetle attractants outside hive, trays to trap emerging pre-pupae, but not use of in-hive traps
- Check hives, especially after catastrophic event, e.g. extreme temperatures, pesticides exposure etc. for SHB

NATIVE HIVE BEETLES, SAP BEETLES *Carpophilus* spp., *Brachypeplus* spp.

- Same family as small hive beetle, but smaller and more elongated
- Scavengers, feeding primarily on pollen stores
- Not major pests

Native sap beetle from hive

POLLEN MITES

- Mites are common inhabitants of nests of colonial and solitary bees, including stingless bees
- Most common are scavenger pollen feeders, such as *Tyrophagus* spp. (stored product or mould mites), or generalist predatory mites which feed off them or use pollen as an alternative food source
- They rarely become a problem and should be regarded as part of the tapestry of life in bee nests
- However, some can cause allergenic responses in susceptible people

Tyrophagus putrescentiae in a stingless bee colony

PREDATORS/PARASITES OF FORAGING STINGLESS BEES OUTSIDE HIVES

- **Native predatory sand wasp**
- **Stingless bee braconid wasp parasitoid**
- **Ants**
- **Spiders**
- **Vertebrates**

PREDATORY SAND WASP *Bembix* spp.

- Hover around hive entrances, and capture emerging bees
- Most species are said to prefer male bees
- Take bees back to their nest in soil to feed their larvae
- No real management strategy, other than to swat them or capture them with sweep nets from hive entrances, but unlikely to threaten hive viability


Australian Stingless
Native Bees


Australian Stingless
Native Bees

SPIDERS

- Predate on foraging bees in flowers or webs near hives
- Minor problem

Flower spider
(Image: David Gray, Australian Museum)

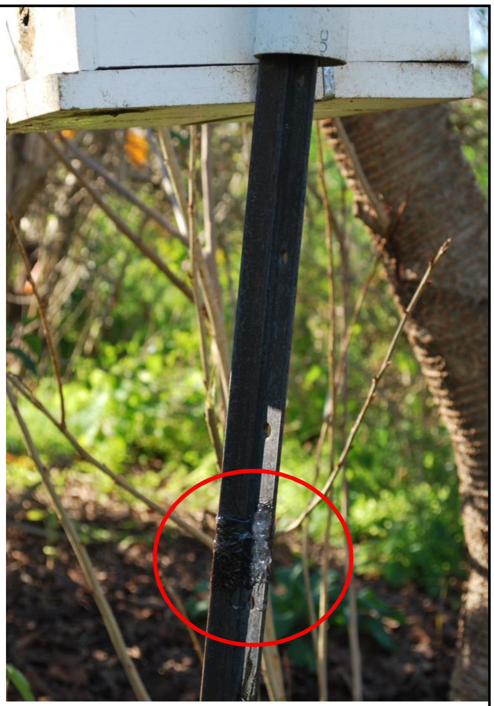
Stingless bees in web near hive entrance WSU

VERTEBRATES

- Range of vertebrate species that predate on pollinators in general, including stingless bees
- These include birds, reptiles and amphibians
- However, minor pests, rarely impact stingless bee colonies

Cane toad waiting for a tasty morsel to emerge
Image: Russell Zabel

Tangle-Trap® on hive posts to restrict ant movement



Taping and locking hives after manipulation (such as splitting) to prevent syrphid and phorid attack

Many stingless bees modify their hive entrances to defend colonies against predators and parasites.
T. carbonaria **reducing entrance to better protect it**

Austroplebeia australis **with full curtain and partial curtain at entrance at night and times of inactivity** (Megan Halcroft)

Stingless bees in tropical areas often modify their entrances especially to protect against weaver (green) ant and other aggressive species

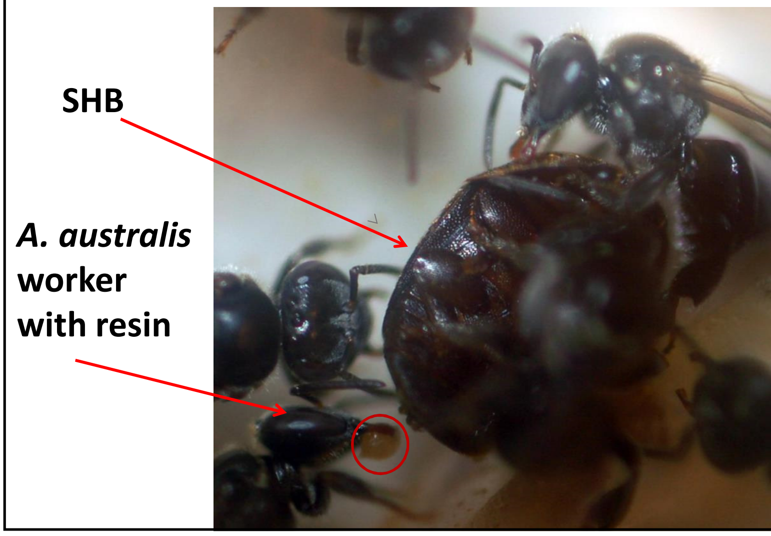
Tetrigona apicalis (Malaysia)

Tetragonula iridipennis (India)

Propolis plug inserted as management practice to reduce hive entrance size after hive manipulation

STINGLESS BEES CAN DEFEND WITHOUT STINGS

Immobilising and imprisoning SHB (Halcroft *et al.* 2011)



Small hive beetle imprisoned

SHB larva attacked by *A. australis* workers

DISEASES OF STINGLESS BEES

- December 2012, Jenny Shanks first observed symptoms in hive of *Tetragonula carbonaria* on WSU Hawkesbury campus, Richmond NSW
- Recorded a range of symptoms in larvae, brood, hive structures and bee behaviour
- SYNDROME: a condition characterized by a set of associated symptoms (but colloquially called **Shanks disease** or **Shanks Brood Disease** [SBD])
- Isolated, identified and confirmed pathogenicity of the bacterium *Lysinibacillus sphaericus*

- Confirmed the causal agent in other *T. c.* hives and also in *Austroplebeia australis* located >20km from WSU.

In common with AFB
- Spore-forming, Gram+ rod
- Infects larvae
- +ve matchstick test
- Isolated from hive stores

Different to AFB
- +ve catalase test
- Toxin not yet detected

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Short Communication

First confirmed report of a bacterial brood disease in stingless bees

Jenny Lee Shanks [a,b], Anthony Mark Haigh [a], Markus Riegler [c], Robert Neil Spooner-Hart [a,c,*]

[a] School of Science and Health, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia
[b] Plant Health Australia, Phipps Close, Deakin, ACT 2600, Australia
[c] Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia

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ABSTRACT

Susceptibility to brood pathogens in eusocial stingless bees (Meliponini), alternative pollinators to honey bees, is unknown. Brood losses in managed colonies of the Australian stingless bee, *Tetragonula carbonaria*, were studied over 20 months. We isolated a disease-causing bacterium, *Lysinibacillus sphaericus* (Firmicutes, Bacillaceae), from worker and queen larvae, brood cell provisions and honey stores. Pathogenicity experiments confirmed this bacterium as the causal organism. It took 22 days from infection to first appearance of brood disease symptoms. This is the first confirmed record of a brood pathogen in stingless bees.

1. Introduction

Brood diseases are of concern for beekeeping because of the effects they have on hive population numbers. Control measures can include antibiotics; however, bacterial resistance is common. *Apis mellifera* can be infected with a number of diseases caused by pathogenic bacteria and fungi (Aronstein and Murray, 2010; Genersch, 2010; Genersch et al., 2010) whereas stingless bees appear to have few brood diseases. The only report is by Kerr (1948), who noted diseased pupae in the Brazilian stingless bees *Melipona quadrifasciata* and *Melipona bicolor bicolor*. *Bacillus paraalvei* was identified in 1957 as the possible causal organism (Nogueira-Neto, 1997). However, at the time only microscopic investigations were performed without any description of pathogenicity, symptoms of the infection or molecular diagnostics. Apart from this, no other reports have been published. Prior to the current study, there were no recorded cases of brood disease in Australian stingless bees.

The Australian stingless bee, *Tetragonula carbonaria*, has been reported to be an effective crop pollinator (Dollin et al., 1997), due to its generalist foraging behaviour (Slaa et al., 2006). *Tetragonula carbonaria* is the most commonly kept (Halcroft et al., 2013) and widespread species of stingless bee, distributed from the warm tropical areas of coastal Queensland (QLD) (Cape York, 16°S, 145°E) to the temperate areas of southern New South Wales (NSW) (Bega, 36° 40.27'S, 149° 50.34'E), and has a flight temperature range between 18 and 35 °C (Rayment, 1935). We report the first detection and identification of a bacterial brood disease, discovered in a *T. carbonaria* hive showing brood losses from December 2012. Over a 20-month observational period starting from March 2013, a further seven colonies showed symptoms of infection.

2. Materials and methods

Tetragonula carbonaria colonies were kept at the Hawkesbury campus apiary, Western Sydney University in Richmond (33° 36.42'S, 150° 44.44'E), NSW, Australia, within their natural range. They were originally sourced from south-east QLD (Australian Stingless Native Bees, Hatton Vale). Four colonies arrived at the apiary in March 2011, and appeared healthy. From then to the first symptoms of disease they experienced normal environmental conditions, with ambient temperatures ranging between 1.5 and 37 °C. However, in December 2012 (after 18 months), upon opening, one hive showed atypical spiral brood structures containing discoloured larvae. The hive was subsequently assessed for its internal colour, structural appearance of the brood, texture of storage pots, involucrum appearance and coverage, formation of brood nest, worker population, in-hive worker behaviour, and colony odour. All brood cells were removed from the colony, and any larvae showing discolouration or fluid appearance in opened cells were separately placed into 1.5 mL sterile Eppendorf tubes. Samples were processed under aseptic conditions, using procedures reported for diagnosis of *Paenibacillus larvae*-infected honey bee larvae (World Organisation for Animal Health, 2013). Individual

* Corresponding author at: School of Science and Health, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia.
E-mail addresses: r.spooner-hart@uws.edu.au, r.spooner-hart@westernsydney.edu.au (R.N. Spooner-Hart).

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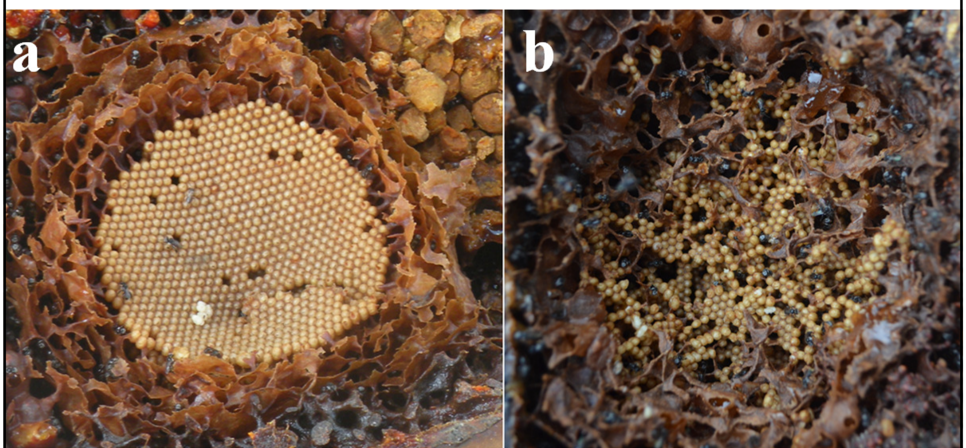
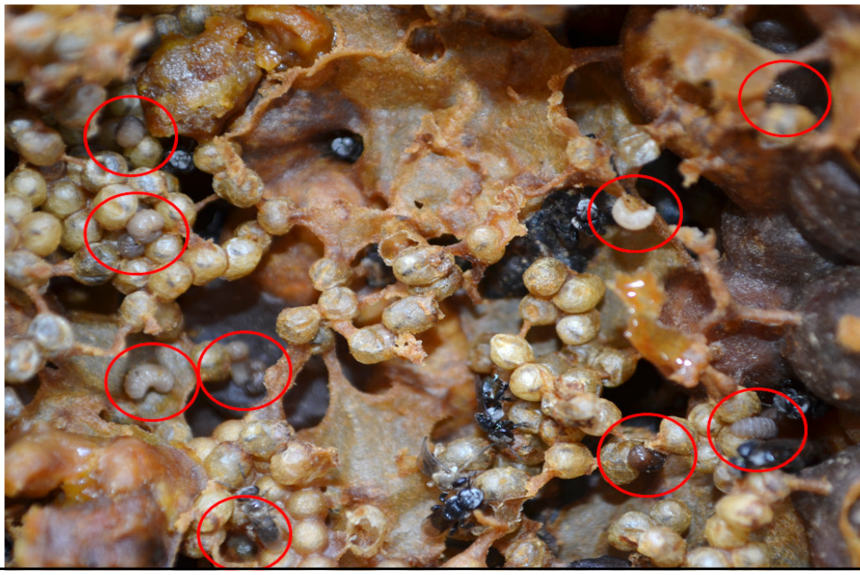
HEALTHY (a) AND UNHEALTHY (b) BROOD IN *T. carbonaria* hive (Shanks *et al.* 2017)



SYMPTOMS OF INFECTED LARVAE (Shanks *et al.* 2017)

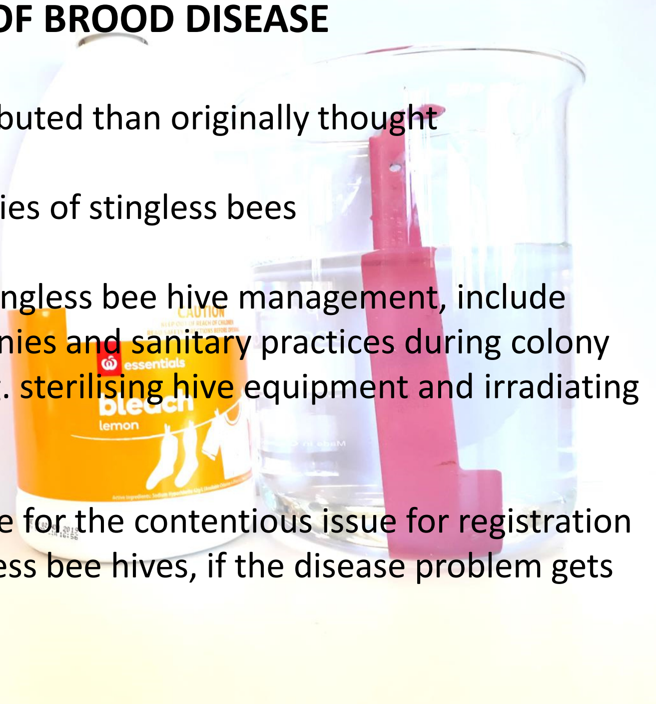
Unhealthy larvae (indicated by red circle) detected and removed from brood cells and deposited on surrounding structures (Jenny Shanks PhD thesis)



T. carbonaria workers taking out the garbage from trash piles inside the hive (this includes infected larvae)

Brood cells completely dismantled after contents have been removed (Jenny Shanks PhD thesis)

IMPLICATIONS OF BROOD DISEASE



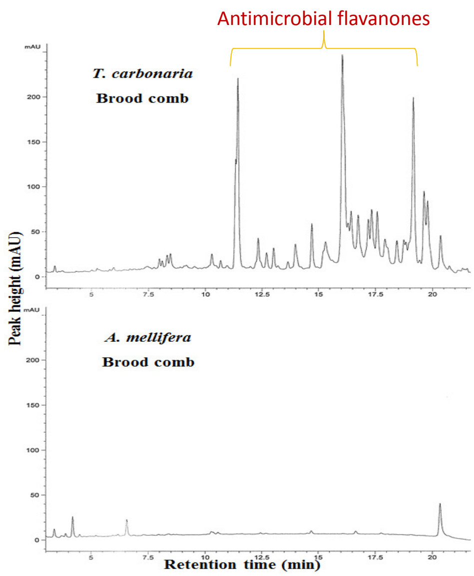
More widely distributed than originally thought

Detected in 3 species of stingless bees

Implications for stingless bee hive management, include movement of colonies and sanitary practices during colony manipulations (e.g. sterilising hive equipment and irradiating dead hives)

May support a case for the contentious issue for registration of managed stingless bee hives, if the disease problem gets worse

Comparison of brood comb composition (LC-MS) between *T. carbonaria* and *A. mellifera,* 2015 (Jenny Shanks PhD thesis)

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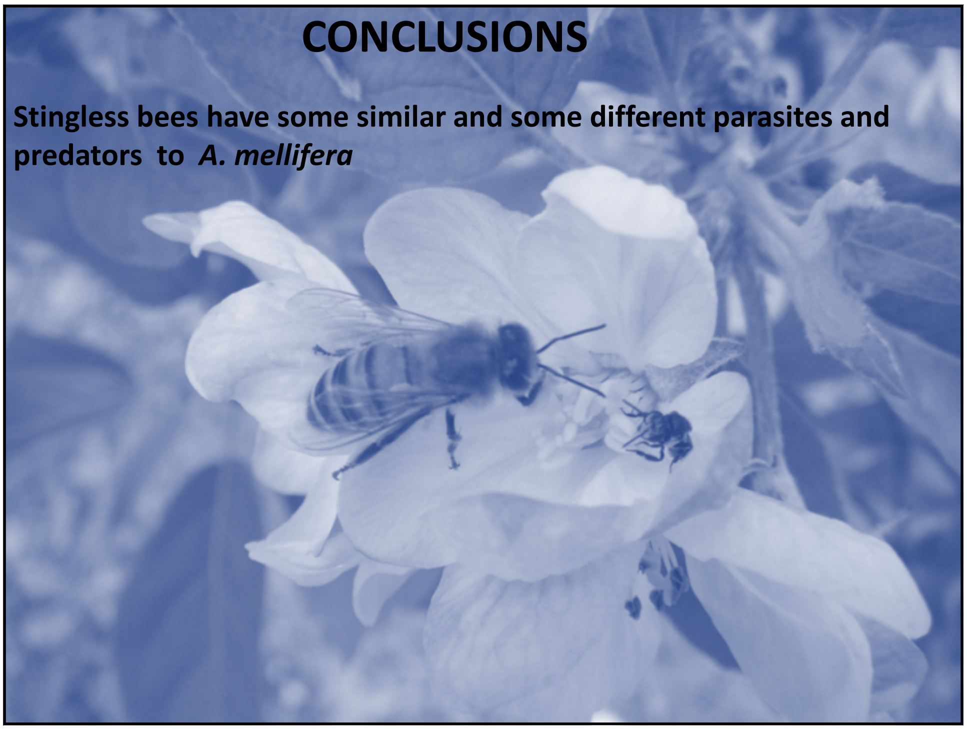
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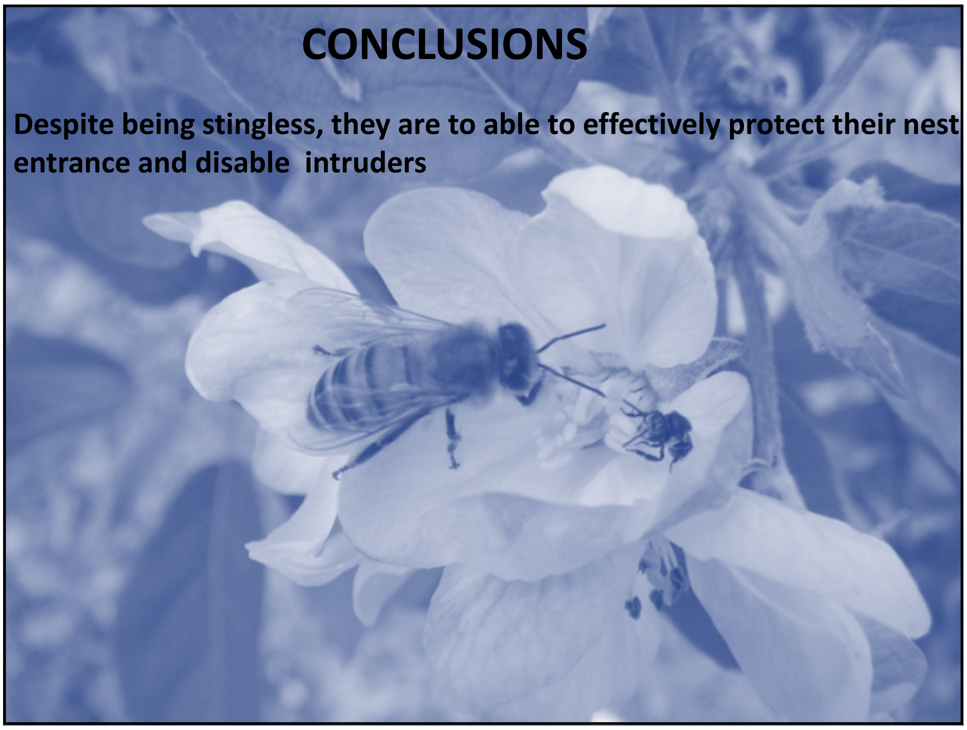
Maybe also because greater incorporation of antimicrobial plant resins into their hive structures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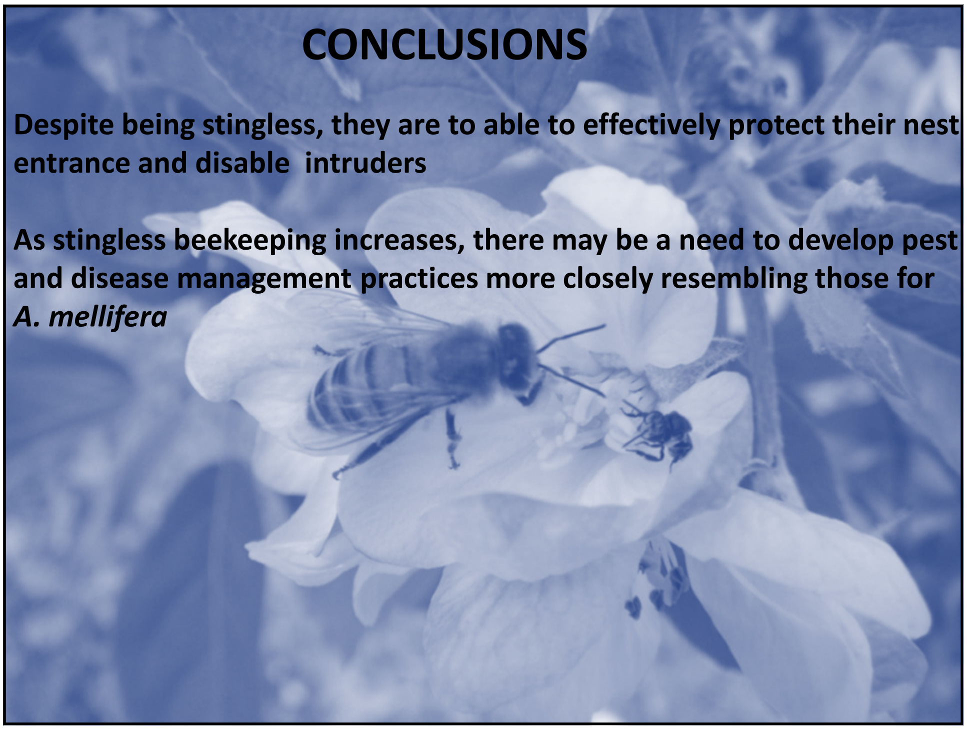
Despite being stingless, they are to able to effectively protect their nest entrance and disable intruders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Despite being stingless, they are to able to effectively protect their nest entrance and disable intruders
As stingless beekeeping increases, there may be a need to develop pest and disease management practices more closely resembling those for *A. mellifera*


THE END