How to Recognise the Different Types of Australian Stingless Bees

Third Edition — PDF eBook

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How to Recognise the Different Types of Australian Stingless Bees

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by Dr Anne Dollin
Australian Native Bee Research Centre

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Booklet 4

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All photographs and drawings are by the author, except where otherwise acknowledged in the text.

FRONT COVER
Left: an Australian *Tetragonula* Stingless Bee worker. **Right**: an Australian *Austroplebeia* Stingless Bee worker. Photographs by Anne Dollin.

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Preface to the Third Edition

We are excited to be able to present to you, in this Third Edition of Booklet 4, a complete guide to identifying the Australian stingless bees. It has taken us 35 years to collect the information needed to write this booklet! Our research into the Australian stingless bees was finally completed late last year.

How it all started

It all started back in 1980 when I began researching our stingless bees for an honours project at Macquarie University. My then boyfriend, Les, and I became fascinated with the Australian stingless bees. However, we soon realised that their species names were in chaos and there was no usable key to the species.

It is very important to be able to identify our stingless bee species, so that we can properly understand these bees, protect them and use them effectively in agriculture. We said, ‘Wouldn’t it be great to sort out all the names of the Australian stingless bees!’ We had no idea where that would lead!

Fifteen different names had been given to our Australian stingless bees by early entomologists between 1854 and 1932. To study these varieties we would need to visit each of the places where the bees had originally been collected, including remote localities in the Kimberley, Arnhem Land, Central Australia and Cape York.

Right from the start we decided to collect samples from nests wherever we could, rather than from flowers. This allowed us to see the variation within the nests and be sure that males belonged to the same species as the females.

Later studies showed how important this decision was. In the *Austroplebeia* for instance, we found that the bees’ colouring varied a great deal. In just one nest, you could find bees that varied so much in colour that they could have come from two or three different species. We could never have understood the subtle differences between these species if we had not made the effort to collect samples from nests.

It was, of course, very time consuming to find nests wherever we went. This is where Les was my secret weapon! He is a superb bushman and became very skilled at finding the many different types of stingless bees in remote parts of Australia.

Our native bee safaris

Planning done, we began our collection work in a series of major native bee safaris. Our research was entirely self funded and we had to save up six weeks of recreation leave from our jobs to do each collection trip.

—For our honeymoon we studied the stingless bees of Cape York Peninsula in far north Queensland. This was a honeymoon with a difference – not too much sipping champagne beside a pool! Instead we set off in our Landcruiser equipped with a microscope, extension ladder and jerry cans of emergency fuel.

—In 1985 we visited the Hamersley Ranges and the Kimberley in Western Australia. We travelled right up to Kalumburu at the top of the Kimberley studying the stingless bees.

—In 1987 we visited Arnhem Land in the Northern Territory to see Port Essington, where *Austroplebeia essingtoni* was first collected. Port Essington was the remote site of an early settlement prior to Darwin.

—In 1990 we trekked right across Cape York to the Gulf of Carpentaria and back looking for a male bee we urgently needed for our *Tetragonula* revision paper. We finally found it at Cooktown: a whole safari for just one bee!

—In 1996 we visited Central Australia to find out about two *Austroplebeia* species collected there in the early 1900s.

—In 1998 we searched western Cape York Peninsula for a minute *Tetragonula* variety called the ‘Mosquito Bee’.

—And in 2012 we travelled to the Daintree in northern Queensland to study a newly-identified population of *Austroplebeia cincta* bees.

Newly-weds, Anne and Les Dollin, about to set out on a six week stingless bee collection trip to Cape York – as a honeymoon!

Sorting out the species names

However, the field work was only part of the job. We ended up with samples from 192 *Tetragonula* nests and 177 *Austroplebeia* nests, from throughout the range of these bees in Australia. Now hundreds of hours of microscope work were required, back in the lab, to analyse these specimens and sort out the species names. Fortunately we received some expert help with this.

Professor Shôichi Sakagami of Hokkaido University in Japan helped us with the *Tetragonula* analyses. He was an expert on the *Tetragonula* species right through South-east Asia. Based on his analyses and our nest and distribution data, we jointly published a paper on the *Tetragonula* species names in 1997. An additional *Tetragonula* species was described in 2004 by Dr Pierre Franck, based on a DNA study.

Our stingless bee revision paper co-authors. Left: the late Professor Shôichi Sakagami. Right: Dr Claus Rasmussen.

The *Austroplebeia* species proved to be far harder to sort out. It was not until we did some collaborative high-tech analyses with Dr Megan Halcroft of the University of Western Sydney and her colleagues in 2009–2011, that we began to see the underlying relationships between the groups. I then embarked on a three year intensive microscopic analysis of our entire *Austroplebeia* collection, that included the discovery of a new species in the Northern Territory. Dr Claus Rasmussen of Aarhus University in Denmark contributed data he had collected on the original *Austroplebeia* specimens in museums in Europe and Australia. Together with Claus, we finally published a paper on the *Austroplebeia* species names in 2015.

Our 35 year study of the Australian stingless bee species has been completed. Eleven valid species of stingless bees are now recognised in Australia and we understand how to distinguish these species. We are pleased to present to you the findings of this research in this third edition of Booklet 4: *How to Recognise the Different Types of Australian Stingless Bees*.

Dr Anne Dollin,
Aussie Bee, 2016.

Scenes from our native bee safaris. Clockwise from top left: Les Dollin sampling stingless bees from a nest in the Gulf of Carpentaria; Anne Dollin examining bees from another nest in Arnhem Land from the top of the Landcruiser; Our faithful blue 'troop carrier' tackling a river crossing during a native bee safari on Cape York.

Come and Meet the Family

The Australian stingless bees are some of our most interesting and well known native bees. They are useful crop pollinators and produce delicious 'Sugarbag' honey. They are safe and fascinating to watch for young and old. So thousands of hives are now kept by backyard beekeepers, farmers and schools.

Australia has eleven known species of stingless social bees. All of them are small black bees less than 5 mm long. How can we identify the different species and how can we distinguish the queens, workers and males in a nest?

We at the Australian Native Bee Research Centre have spent decades studying the species of Australian stingless bees. We conducted ten major field expeditions to remote areas of Australia and spent hundreds of hours doing microscopic measurements of the specimens we collected.

Come with us now and meet the family as we introduce to you in detail the Australian species of stingless social bees.

Les Dollin of the Australian Native Bee Research Centre hunting for stingless bees in Central Australia

First a Broader Look at Australia's Bees

The stingless bees, that are the main topic of this booklet, are social bees. They live in large colonies that have a queen bee, males and hundreds or even thousands of worker bees. The queen bee normally lays all of the eggs in the nest; whilst the workers are sterile female bees that do most of the work in the nest. However, fewer than 1% of Australia's native bee species are social bees.

Australia has about 1,700 species of native bees. The other 99% of our species are solitary and semi-social native bees.

— Solitary native bees live individual lives. Each female bee raises her young in her own nest burrow and the mother bee generally dies before her offspring hatch.

— In semi-social native bees, two or more females raise their young in a common nest and they share some duties inside the nest.

Almost all of Australia's solitary and semi-social native bees can sting, though most are too small to effectively sting humans. Many nest in burrows in the ground, whilst others nest in small holes in timber or inside pithy stems. They vary widely in shape, size and colour.

In addition to our native bees, we have a number of bee species that have been introduced from overseas. The commercial honeybee (*Apis mellifera)* is the bee most frequently seen in our gardens. This bee was introduced from Europe in 1822 and now is used throughout Australia for crop pollination and honey production. We also have some species that were accidentally introduced, including European bumblebees (*Bombus terrestris)* in Tasmania, Asian honeybees (*Apis cerana*) in northern Queensland and South African Carder Bees (*Afranthidium repetitum*) in Queensland, New South Wales and Victoria.

A solitary resin bee (12 mm long) sealing her tiny nest that she has built inside a hole drilled into a block of timber. She is working with a mixture of resin and chewed leaf material.

To accurately identify bees, you need to look at tiny details of their colouring, hair and body shape with a strong magnifying glass or a microscope. However, there are photos and videos of many of these bees on Aussie Bee website (**www.aussiebee.com.au**) as well as a field guide that describes some common species. One of our other ebooks, *Introduction to Australian Native Bees* (see page 32), also explores Australia's diverse bee species.

Australian Stingless Bees

An Australian Tetragonula carbonaria stingless bee

Our Australian stingless bees, that are the main focus of this booklet, are found in the warm northern and eastern parts of our country. They live in large social nests with queens, males and hundreds of workers, usually inside hollow trees. They are small black bees up to about 5 mm long.

Our stingless native bees can be distinguished from our other native bee species by a special structure called a *pollen basket* or *corbicula* on the hind legs of the worker bees. This is a smooth dish-like structure (see diagram below) which they use for carrying pollen. The only other bees in Australia which have pollen baskets are the introduced honeybees and bumblebees. However, honeybees (10–12 mm long) and bumblebees (8–25 mm long) are much larger than our stingless bees (3.5–4.5 mm long).

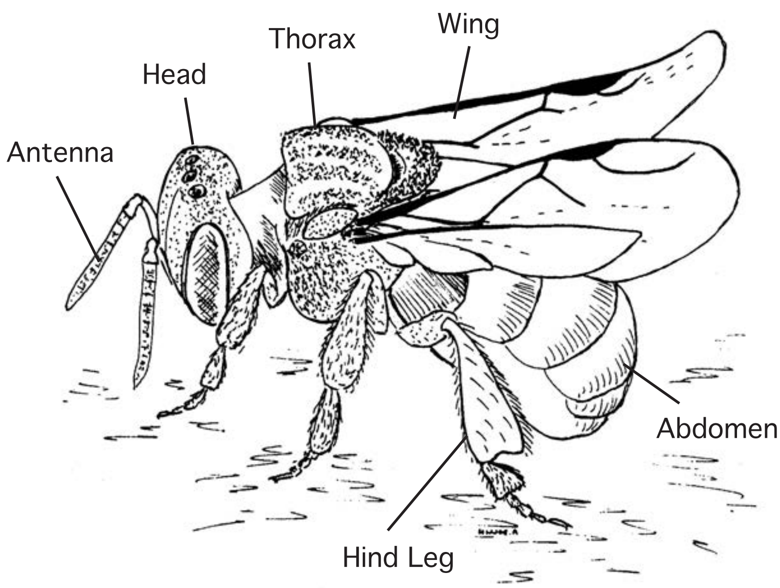
Overseas, several hundred species of stingless bees are found in tropical Asia, Africa and America. Stingless bees overseas vary widely in shape, colour and size. Some species are larger than the commercial honeybee, whilst others are just a few millimetres long. Some build nests with entrance tunnels up to one metre long; some build nests deep underground; others build exposed nests in trees, covering them with their own resinous sealing layers.

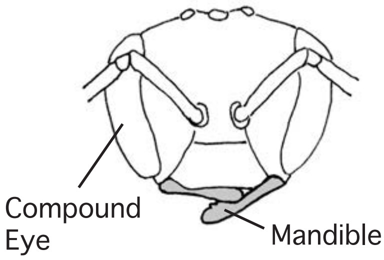
In contrast, our Australian species are much more uniform in appearance and behaviour and so, unfortunately, our different species are much harder to tell apart. However, by using the charts on the following pages, you should soon be able to recognise most species of our Australian stingless bees.

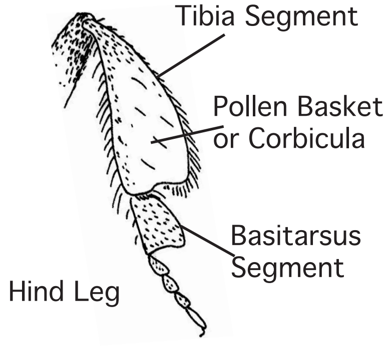
Learn to Identify The Bee's Knees
(and other bits)

First, we need to understand a few things about a bee's body. A bee has its skeleton on the outside. Its body is covered with hard plates. From the time a bee emerges from its brood cell, these plates do not change in shape or size. (Ever heard of an overweight bee?) So we can distinguish many of the species by looking at the shape and size of the bee's body parts. The names of some important parts are shown in the diagrams below.

To identify a stingless bee species you will need to study some of these body parts with a strong magnifying lens or microscope. The best sort of microscope for this is a dissecting microscope that provides a magnification of about 15 to 40 times. You can keep a bee still, whilst you briefly study it, by placing it in a jar in the refrigerator (not the freezer!) for a few minutes until it falls asleep. Then place it on a frozen icebrick wrapped in a tea towel under your microscope or magnifying lens. When you are finished, allow the bee to warm up to room temperature again and it will normally recover and fly off.







The Three Castes in a Stingless Bee Nest

[Everything You Wanted to Know about Sexes... but were afraid to ask!]

In every social stingless bee nest, there are three castes or types of bees.

— one mature **queen** and often some virgin queens (fertile females that can lay eggs);
— hundreds of **workers** (sterile females); and
— usually some **males**.

In Australian stingless bees, the mature queen can be quite easily spotted because of her large abdomen. However, the workers and males can be so similar that you might need your strong magnifying lens again to recognise them.

Queen bees

The mature queen can be recognised by her long, often creamy coloured abdomen, which is full of eggs. Even newly emerged virgin queens have longer abdomens than worker bees have. However, once a queen bee begins laying eggs, her abdomen becomes so large that she can no longer fly. She is usually found in the brood area of the nest.

Stingless bee queens do not collect pollen in the field. So they also do not have the pollen basket structure for carrying pollen on their hind legs that worker bees have.

An Austroplebeia cincta queen bee

Workers and males

In our Australian *Austroplebeia* species, the males are reasonably easy to spot. They tend to be more slender than the worker bees and to have much brighter colour markings. They usually have yellow on the legs and abdomen, and the markings on the thorax are usually heavier and brighter.

Unfortunately our *Tetragonula* species lack colour markings, so their workers and males are much more difficult to distinguish. You have to rely on the tiny structural differences between males and workers described below.

The structural differences between worker bees and males in stingless bees relate to the different roles these bees play in the life of the colony:

— Worker bees do most or all of the construction work within a nest, using their mandibles to manipulate the resins and build the intricate nest structures. They are also responsible for defending the nest and may use their mandibles to nip an intruder. So worker bees have larger, stronger mandibles, than the males do.

— Worker bees forage outside the nest for food and nesting materials. So they have special smooth plates on their hind legs, used for carrying pollen and resin back to the nest. These plates are called *pollen baskets*.

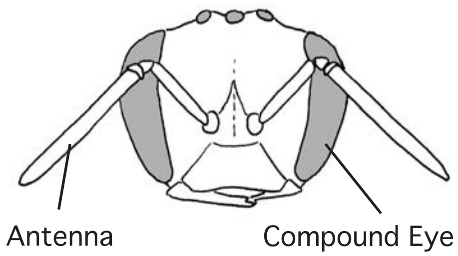
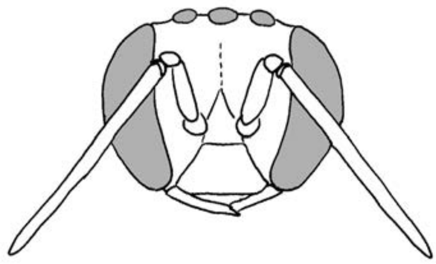
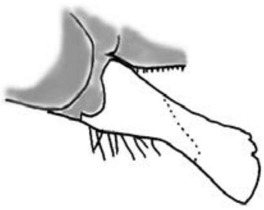
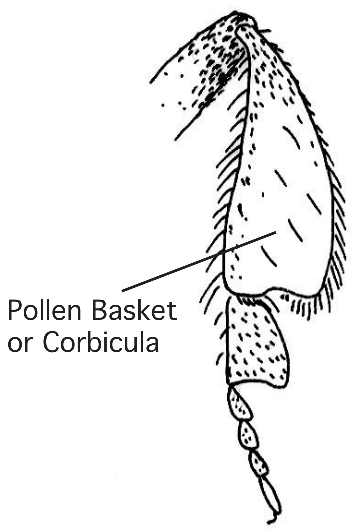
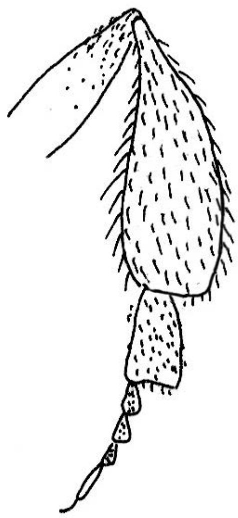
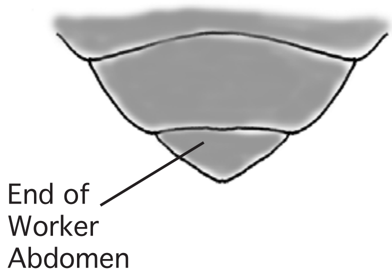
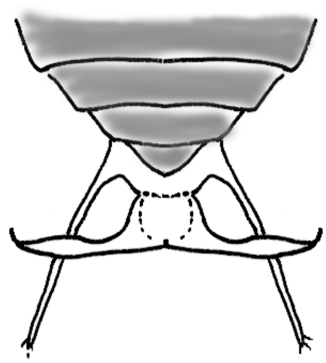
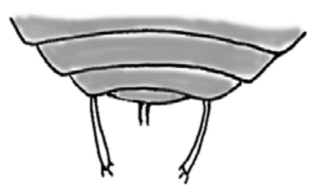
— Males must desperately compete with one another for the chance to mate with a virgin queen during her mating flight. In the stingless bees, only one male mates with each queen bee. They need excellent vision and scent detection to locate the virgin queen in the swarm. So they are equipped with particularly large eyes and long antennae.

A stingless bee worker disposing of a dead nestmate. Photograph by Peter O.

— The sexual organs of the male include hardened clasper-like structures that help him latch onto a virgin queen during a mating swarm and successfully mate with her in mid air. In a dead male, these hardened parts can often be seen protruding from the tip of his abdomen.

The differences between workers and males are summarised in the following chart:

Differences between Workers and Males

	Workers	Males
Antennae Males have longer antennae **Compound Eyes** Males have larger compound eyes		
Mandibles Worker bees have larger, stronger mandibles		
Hind Legs Worker bees have pollen baskets on their hind legs, for transporting pollen and resin. These are concave, polished plates, with rows of long bristles around them. The hind legs of the male are rounded and more evenly covered with fine hairs.		
Hardened male sexual organs The genitalia may be seen protruding from the end of the abdomen of a dead male bee. They may be fully protruding (a) or only the tips may be visible (b)	*(These diagrams show the top view of the end of the abdomen.)* 	(a)  (b) 

The Two Australian Stingless Bee Groups: *Tetragonula* and *Austroplebeia*

Today our Australian stingless social bees are classified in two genera or groups called *Tetragonula* and *Austroplebeia*. As shown in the charts on pages 6–7, our *Tetragonula* and *Austroplebeia* species are very distinct, with differences in body shape, colouring and nest structure. However, it took over 150 years to work out the classification we use today.

Why are they now called *Tetragonula* and *Austroplebeia*?

In the 1800s our Australian stingless bees were all classified in one genus named *Trigona*. In 1961 the Brazilian stingless bee expert, Professor JS Moure proposed a classification(1) that divided the stingless bees of Asia, Australasia and Africa into many different genera. However, most Australian reference books such as The *Insects of Australia*(2) followed an alternative classification system used by the American expert, Professor CD Michener.

In 1990 Professor Michener, divided the Australian stingless bees into two genera: *Trigona* and *Austroplebeia*.(3) However, the *Trigona* genus was a huge one, with over 100 species worldwide. A major DNA study in 2007(4) showed that the *Trigona* bees in our region were sufficiently distinct to deserve their own genus name. So in 2013 Professor Michener reviewed all of the world's *Trigona* species to sort this out.(5) He placed our Australian *Trigona* species, along with 30 species from other countries in our region, into the genus *Tetragonula*.

Austroplebeia and *Tetragonula* were two of the genus names that had been proposed by Professor Moure in his 1961 classification.(1) So the names we use today for our stingless bees combine insights from these two great international bee experts. Read more: **www.aussiebee.com.au/tetragonula–name–change.html**

Sorting out the species names of our stingless bees

When my husband, Les, and I first started studying native bees back in 1980, the species names of our stingless bees were in great disarray. Fifteen different names had been given to our bees between 1854 and 1932 by early entomologists. Many of the names were based on just one or two bee specimens that had been collected decades earlier and placed in overseas museums. There was very little clear information on how to recognise the species or how to distinguish them from other species. A thorough review of the species names of our stingless bees was greatly needed.

Anne Dollin studying a nest in the Northern Territory in 1987. This turned out to be a new species that we called Austroplebeia magna.

Les and I decided to try to sort all of this out. Little did we guess that this task would take 35 years to achieve! We set out on ten major native bee safaris across Australia. We visited each of the localities where a species had been named and did detailed studies of the stingless bees we found there: the workers, males and queens and their nest structures. Eventually we built up a huge set of information, based on samples from well over 300 *Tetragonula* and *Austroplebeia* nests.

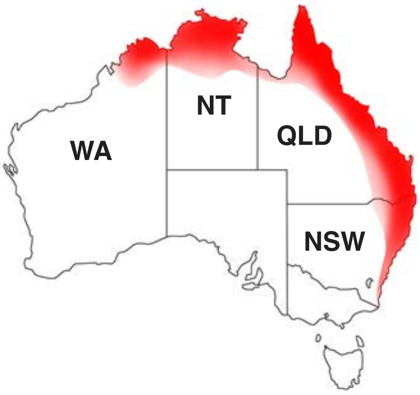
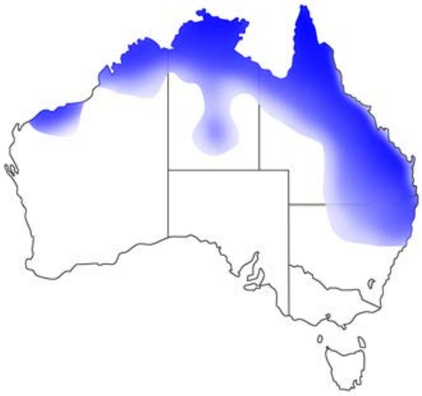
In 1982, a Japanese stingless bee expert, Professor Shôichi Sakagami, offered to collaborate with us. Together with him, we published a paper in 1997 sorting out the species names of the *Tetragonula* stingless bees.(6)

However, the *Austroplebeia* proved to be much harder to distinguish. Then, in 2010, we had the chance to work with Dr Megan Halcroft on a DNA study which provided some valuable insights.(7) Building on this, we did an in-depth analysis of all the *Austroplebeia* samples that we had collected across Australia. Finally with the help of Danish stingless bee expert, Dr Claus Rasmussen, we published a paper in 2015 sorting out the species names of the *Austroplebeia* stingless bees.(8)

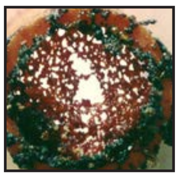
These two papers present detailed formal descriptions of our *Tetragonula* and *Austroplebeia* species, and keys explaining how to distinguish the species. The most important findings from these two papers are explained here in this ebook. For a behind-the-scenes look at how the research was done, including stories from some of our native bee safaris, read Aussie Bee Online article 25: Meet the *Austroplebeia* species. To download your free copy, visit: **www.aussiebee.com.au/abol–current.html**

To find out if your bees are *Tetragonula* or *Austroplebeia*, look at the chart on page 6

The Differences Between *Tetragonula* and *Austroplebeia* Bees in Australia

	Tetragonula	*Austroplebeia*
Distribution	 **Six Known Species** The richest diversity of *Tetragonula* species is in the Cape York area of QLD, where four of the species can be found. The distribution of *T. carbonaria* extends southwards to Bega in NSW – the most southern population of stingless bees in the world.	 **Five Known Species** The *Austroplebeia* can survive colder and more arid conditions than the *Tetragonula*. So populations extend into Central Australia, NT and into the Hamersley Range, WA. One species, *A. cincta*, is also found in New Guinea.
Brood structure inside the nest	 The waxy brood cells that contain larvae (shown above) are oval shaped and open upwards. The cocoons that contain pupae are also oval shaped. The cells are connected together into groups. Some *Tetragonula* species build neat, regular single layer combs, whilst others arrange their cells more loosely in irregular layers. See photographs on pages 10–13.	 The waxy brood cells that contain larvae (shown above) are spherical and can open in any direction. The silken cocoons that contain pupae are oval shaped. With the exception of *A. cincta*, *Austroplebeia* bees arrange their cells irregularly in a cluster, without any comb structure. See photographs on pages 26–27 and 29–31.

Nest entrance closures



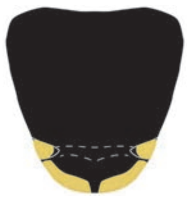
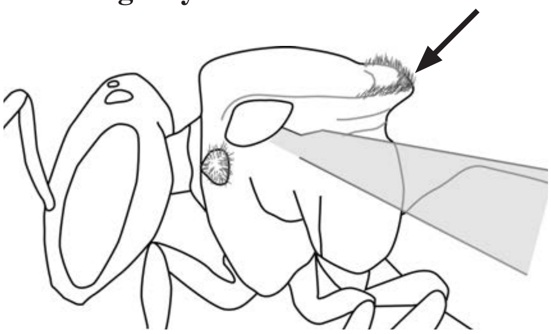
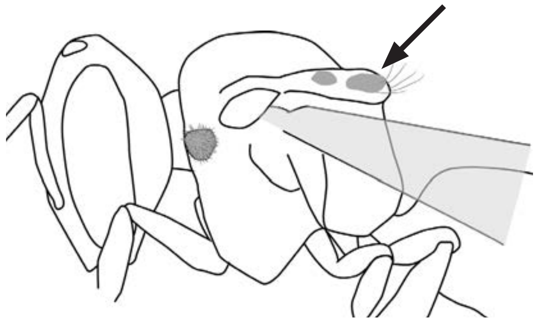
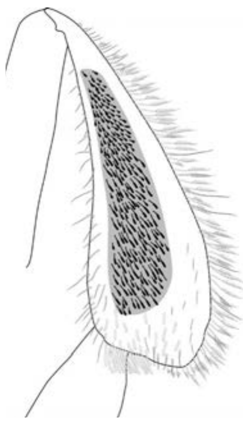
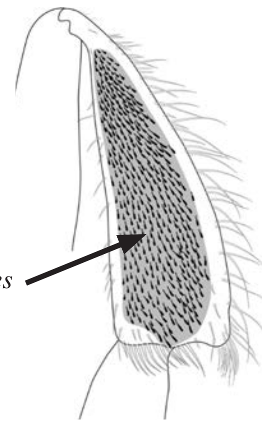
In southern Queensland and in New South Wales, a simple way to distinguish the local species of *Tetragonula* and *Austroplebeia* is to examine the nest entrances at night or in periods of cold weather. In *Tetragonula carbonaria* and *T. hockingsi*, the nest entrance is left open. In contrast, in *Austroplebeia australis* and *A. cassiae*, the nest entrance is closed up with a fine lacy curtain of resin blobs (shown in photo).

However, some species in other parts of Australia behave differently. For instance, *Tetragonula clypearis* and *T. sapiens* bees may close their entrances at night (see photograph on page 14), whilst *Austroplebeia cincta* bees may leave their entrances open.

...Continued on page 7

The Differences Between *Tetragonula* and *Austroplebeia* Bees in Australia – continued

The following characteristics of the bees themselves provide a more precise way to work out whether your bees belong to *Tetragonula* or *Austroplebeia*:

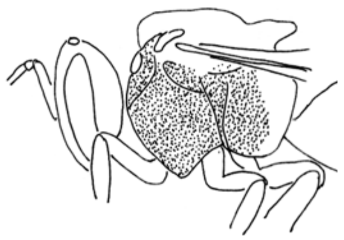
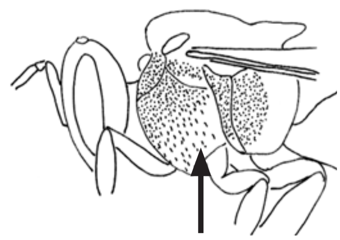
	Tetragonula	*Austroplebeia*
Colour Markings	**Workers have no colour markings on the face or thorax.** The face and thorax of our *Tetragonula* workers are black or very dark brown. They do, however, have dense coatings of white fur on the face and on the side of the thorax, that can make these areas look whitish.	**Workers usually have some colour markings on the face or thorax.** There is nearly always at least a triangular cream or yellow mark on the face between the antennae...  and usually some cream or yellow markings on the thorax — see pages 15–24 for more details. 
Thorax Shape	**The back rim of the thorax juts out quite a long way.** 	**The back rim of the thorax is less protruding.** 
Bristles inside hind leg	**Narrow band of bristles inside hind leg** There is a band of blunt bristles (called the *keirotrichia*) inside the tibia section of the hind leg of the workers. The bristles appear to be used for cleaning the wings. In the *Tetragonula* this band is narrow. 	**Broad band of bristles inside hind leg** In the *Austroplebeia*, the band of blunt keirotrichia bristles inside the tibia is broad. *Keirotrichia bristles* 

If your bees are *Tetragonula*, go to page 8
If your bees are *Austroplebeia*, go to page 15

The Six Australian *Tetragonula* Species

Currently six species of *Tetragonula* are recognised in Australia: *T. carbonaria, T. hockingsi, T. davenporti, T. mellipes, T. sapiens* and *T. clypearis*.[(6), (9)] They fall into two major groups that can be identified by looking at the hair patterns on the side of the thorax. However, apart from that, these species are most easily recognised by their body size, distributions and features of their nest construction, as explained in the following chart:

<table>
<tr><th>Species name & body length of worker</th><th>Distribution</th><th>Hair on side of thorax</th><th>Brood structure</th><th>External entrance</th><th>Nest location</th><th>More details</th></tr>
<tr><td>Tetragonula carbonaria
4 mm</td><td></td><td rowspan="4">EVEN
See diagram below left</td><td>Regular horizontal single-layer spiral.</td><td>Entrance bare or with a coating of resin around it.</td><td rowspan="3">Usually inside hollow trees.</td><td>Page 10.</td></tr>
<tr><td>Tetragonula hockingsi
4.5 mm</td><td></td><td rowspan="3">Many small interconnecting horizontal combs.</td><td rowspan="2">Entrance bare or with resinous deposits around it.</td><td>Pages 10–11.</td></tr>
<tr><td>Tetragonula davenporti
4 mm</td><td></td><td>Pages 11–12.</td></tr>
<tr><td>Tetragonula mellipes
4 mm</td><td></td><td>Often builds a round entrance tube up to 40 mm long.</td><td>Inside hollow trees or wall cavities.</td><td>Page 12.</td></tr>
<tr><td colspan="7"></td></tr>
<tr><td>Tetragonula sapiens
4 mm</td><td></td><td rowspan="2">UNEVEN
See diagram below right</td><td>Brood cells waxed together into rough diagonal or horizontal layers. See page 13.</td><td>Usually builds a round, short entrance tube, up to 20 mm long.</td><td>In cavities in stone walls or buildings, as well as inside hollow trees.</td><td>Page 13.</td></tr>
<tr><td>Tetragonula clypearis
3.5 mm</td><td></td><td>Brood cells roughly arranged in diagonal rows. See page 13.</td><td>Entrance tube may be short and round, or up to 180 mm long, built on the surface of the building or post.</td><td>Common in man-made cavities, such as inside walls, doors and posts, but also nests inside hollow trees.</td><td>Pages 13–14.</td></tr>
</table>

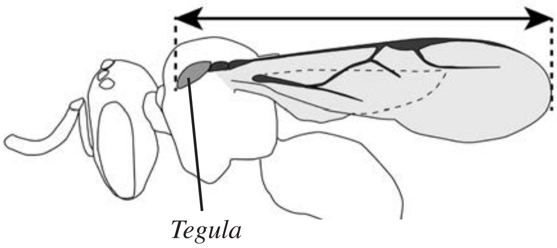
<table>
<tr><th colspan="2">EVEN THORAX HAIR</th><th colspan="2">UNEVEN THORAX HAIR</th></tr>
<tr><td colspan="2">T. carbonaria, T. hockingsi, T. davenporti, T. mellipes</td><td colspan="2">T. sapiens, T. clypearis</td></tr>
<tr><td>The whole of the side of the thorax is covered with even, dense white hair.</td><td></td><td>The areas at the front and the back have dense white hair, but the area in the middle (arrow) has only sparse white hair.</td><td></td></tr>
</table>

Further Identification Tips for *Tetragonula*
— for the technically minded

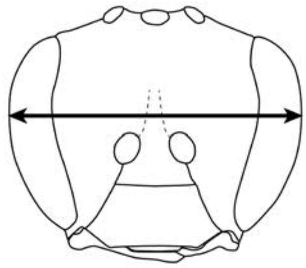
The following is a more detailed method for identifying the *Tetragonula* species using measurements, hair patterns and nest characteristics. For further details, please see our 1997 revision paper.[6]

Instructions for taking measurements on bees using a microscope are given in Appendix 1 on page 34. The following two measurements of the worker bees are used in this method:

Fore wing length (including the *tegula,* which is the small plate covering the hinge of the wing)



Head width



These two measurements are much more useful than a measurement of the bee's overall body length. A bee can expand and contract its abdomen, causing a substantial variation in the length of its body.

The size ranges given in the following tables are based on measurements we did on hundreds of worker bees sampled from nests from across Australia over the past two decades. The size ranges do overlap between the different species. Nevertheless, these size measurements provide the most reliable method of identifying these *Tetragonula* species, other than examining their nest characteristics.

We found that size varies slightly across the distribution of these species. For instance, *T. carbonaria* bees from QLD were a little smaller than those from NSW.

Even different bees from the same nest vary slightly in size. You should measure several worker bees from the same nest and average their scores, to get the most accurate result.

Tetragonula Species Identification Charts

WORKER BEES: with EVEN hair on side of thorax (see diagram on page 8):					
	Distribution	**Fore wing length including tegula**	**Head width**	**Nest has external entrance tunnel**	**Brood structure**
T. carbonaria	NSW	4.3–4.6 mm	1.72–1.81 mm	No	Regular spiral
T. carbonaria	QLD	4.1–4.5 mm	1.71–1.77 mm	No	Regular spiral
T. hockingsi	QLD &NT	4.4–4.7 mm	1.88–1.95 mm	No	Small interlocking combs
T. davenporti	QLD (Gold Coast)	4.3–4.6 mm	1.68–1.76 mm	No	Small interlocking combs
T. mellipes	WA & NT	3.9–4.3 mm	1.72–1.78 mm	Usually	Small interlocking combs

WORKER BEES: with UNEVEN hair on side of thorax (see diagram on page 8):					
	Distribution	**Fore wing length including tegula**	**Head width**	**Nest has external entrance tunnel**	**Brood structure**
T. sapiens	QLD (Cape York)	4.2–4.5 mm	1.76–1.82 mm	Usually	Irregular
T. clypearis	QLD (Cape York)	Less than 3.8 mm	Less than 1.6 mm	Usually	Irregular

More Details About the Six Known Species of *Tetragonula* in Australia

Tetragonula carbonaria

This was the first stingless bee species to be named in Australia. It was described by entomologist, Frederick Smith, in 1854 based on specimens held by the British Museum. The only information Smith gave about where the bee was collected was 'Habitat: Australia'!

T. carbonaria is common on the east coast of Australia from southern New South Wales to Bundaberg, Queensland, but it may also be found as far north as Daintree, Queensland. In New South Wales it is found as far south as Bega (latitude 36.4° S), making it the most southern species of stingless bee in the world. In cooler areas, *T. carbonaria* bees nest in large trees, up to 1.5 m in diameter, that provide vital insulation to help them survive.

Estimated current distribution of Tetragonula carbonaria

The nest entrance of a *T. carbonaria* nest is usually just a simple hole in the timber. Often the timber surrounding the nest entrance is coated with a thick smooth layer of resin that may be black, red or yellow. The brood cells of *T. carbonaria* are arranged in an elegant single-layer horizontal spiral comb. New brood cells are built on the outside edges of the spiral comb.

T. carbonaria is the most common species kept in boxed hives by beekeepers in Australia. It is used for pollinating crops such as macadamias and can also be used for honey production in warm areas.

Left: the resin smeared entrance of a T. carbonaria nest. Centre: the elegant spiral brood comb built by T. carbonaria bees. Right: side view of the layered brood comb built by T. carbonaria.

Tetragonula hockingsi

This species was described by TDA Cockerell in 1929 based on bees collected in Cape York, Queensland. It was named after its collector, Harold Hockings, a naturalist who had presented a detailed early report about *T. carbonaria* and *A. australis* to the Entomological Society of London in 1884.[10]

T. hockingsi is common in eastern Queensland from Cape York to Bundaberg. However, in recent years it has been spreading into more southern areas of Queensland, down to the Brisbane River. Nests have been also found in northern areas of the Northern Territory, however, we think that these bees may differ genetically from the eastern populations.

Estimated current distribution of Tetragonula hockingsi

The entrances of many *T. hockingsi* nests are bare but in some nests a large area around the entrance hole is daubed with blobs of resin and other materials. Some *T. hockingsi* bees will collect fresh paint as a resin substitute and the hardened paint droplets may be seen around the entrance hole and throughout the nest structures.

The brood cells of *T. hockingsi* are arranged in small horizontal combs that interconnect with one another in an irregular pattern resembling cobblestones in a staircase. New brood cells are built on one side of each of the small combs.

T. hockingsi bees look extremely similar to *T. carbonaria* bees but they are usually slightly larger and more aggressive. Their powerful colonies can build huge nests containing thousands of worker bees. Boxed hives of *T. hockingsi* are kept by many beekeepers in northern Queensland and are also used for crop pollination and honey production.

Left: T. hockingsi bees guarding a narrow nest entrance. Centre: this T. hockingsi nest, under attack by green ants, surrounded its nest entrance with a conglomeration of resin and dead ants.
Right: the brood comb of T. hockingsi with the cells arranged in small interlocking combs.

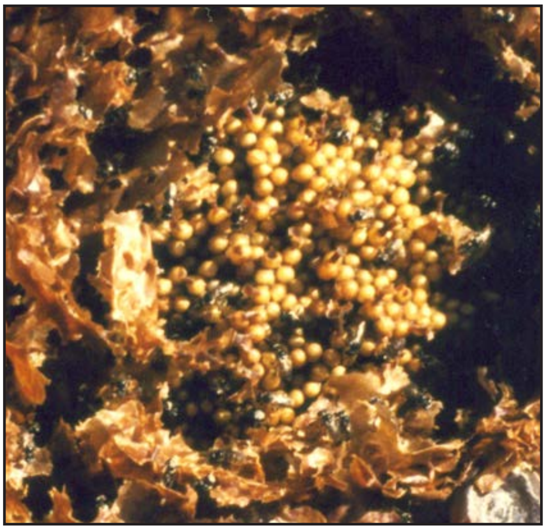
Tetragonula davenporti

T. davenporti was named after one of Australia's most experienced stingless bee keepers, Peter Davenport, who first discovered these unusual bees. This species closely resembles *T. hockingsi*. However, *T. davenporti* bees are fractionally smaller than *T. hockingsi* bees and they are much less aggressive in their behaviour.

The only known populations of *T. davenporti* are found in the Gold Coast hinterland area of Queensland.

Peter Davenport says the entrance holes of *T. davenporti* nests and hives are usually bare of resin. *T. davenporti* builds a brood with small irregular interconnecting combs, very similar in structure to the brood of *T. hockingsi*. However, *T. davenporti* broods and nests are usually much smaller in overall size.

Estimated current distribution of Tetragonula davenporti



Left: an irregular arrangement of cocoons in a T. davenporti brood.
Right: the advancing front of a T. davenporti nest with the cerumen cells arranged in small interlocking combs, very similar to those of T. hockingsi. Photograph by Peter Davenport.

Despite their physical similarities to *T. hockingsi*, Pierre Franck found that these bees were quite different from *T. hockingsi* in their DNA. So in 2004 he described them as a separate species and named them *T. davenporti*.[9]

It is fascinating how difficult it is to distinguish *T. davenporti* from the other *Tetragonula* species using microscopic measurements, even though this species is so different in its DNA. Many bird and mammal species recognise one another using visual features such as colour patterns or body shapes, which are easy for humans to identify. However, stingless bee species mainly recognise one another using scents or pheromones, which humans need special equipment to even detect. The stingless bees, with their highly sensitive sense of smell, have no problem recognising one another using pheromones. However, we poor humans, relying on what we can see through our microscopes, can find the job of recognising stingless bee species extremely difficult!

Tetragonula mellipes

This species was described by Heinrich Friese in 1898 as a honey yellow coloured bee. He gave them the name 'mellipes' which means 'honey footed'!

However, in all known Australian *Tetragonula* species, the adult bees are black. The identity of this mysterious 'honey yellow' species was not unravelled for ninety years.

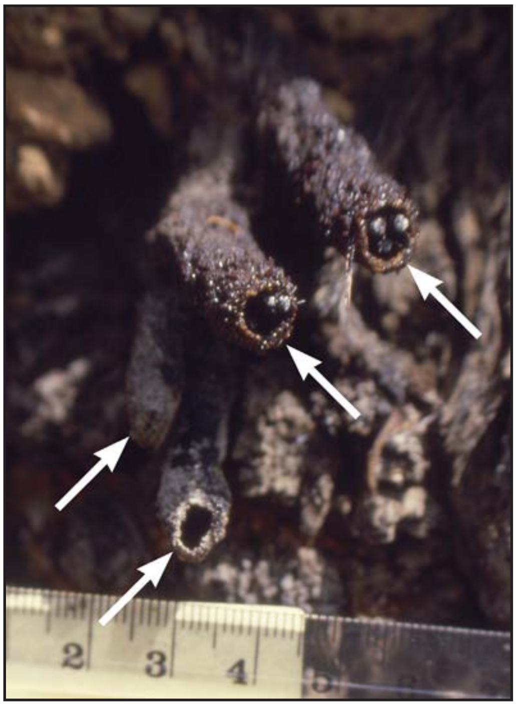
In 1987 we found some nests of black *Tetragonula* bees in Arnhem Land, Northern Territory. Meanwhile in a Berlin museum, our colleague, stingless bee expert SF Sakagami, found some pale brown coloured, ninety year old specimens that Friese had labelled as *T. mellipes*. Using microscopic measurements Professor Sakagami matched these old *T. mellipes* specimens with our modern Arnhem Land bees. The mysterious *T. mellipes* species had been rediscovered!

Estimated current distribution of Tetragonula mellipes

So why were these old specimens pale brown? When stingless bees first emerge from their brood cells they are cream coloured and they do not achieve their mature black colouring for quite a few days. We believe that the original specimens that Friese described as 'honey yellow' must have been recently emerged bees collected from the brood of their nest.

Friese stated that the original specimens of *T. mellipes* were collected in 'South Australia'. At that time, 'South Australia' included the whole region now covered by the Northern Territory and South Australia. We have now found *T. mellipes* bees in many northern areas of Western Australia and the Northern Territory.

Most *T. mellipes* nests build a short resinous tube for their nest entrance. One unusual nest had four parallel entrance tunnels (see below). Their brood cells are arranged in small irregular combs.



Left: an unusual T. mellipes colony with four parallel nest entrance tunnels in the Northern Territory. One tunnel has been closed off at its tip but the other three tunnels are open.
Right: side view of the brood comb of a T. mellipes nest with the cocoons arranged in small irregular combs.

Tetragonula sapiens

This species was described by TDA Cockerell in 1911 based on specimens collected in the Solomon Islands.

Our Australian bees of this variety were originally thought to belong to a very similar species called *Tetragonula laeviceps*. However, in 1997[6] it was discovered that our bees actually belong to the *T. sapiens* species of New Guinea, Indonesia, the Solomon Islands and the Philippines.

In Australia, *T. sapiens* is found on Cape York Peninsula and the east coast of far north Queensland. Its nests are often found close to those of *T. clypearis*. However, these two species can be readily distinguished, because *T. sapiens* workers and males are distinctly larger than those of *T. clypearis*.

Estimated current distribution of Tetragonula sapiens

T. sapiens bees usually choose cavities in stone walls or in hollow trees for their nests. They almost always build a short tunnel for their nest entrance. Their brood cells are arranged in irregular horizontal or diagonal layers, reinforced with coatings of their cerumen building material.

Left: the entrance tunnel of a T. sapiens nest that is in the cavity inside a stone wall. Centre: this T. sapiens nest is inside an underground galvanised pipe – entrance tunnel indicated with an arrow. Right: side view of the brood of a T. sapiens nest with cerumen cells waxed into irregular layers at the top and rough rows of cocoons below.

Tetragonula clypearis

This is the smallest *Tetragonula* species in Australia. It was described by Heinrich Friese in 1908, based on bees from Indonesia.

Australian bees of this type were originally called *T. wybenica*. They were named after 'Wyben', the Aboriginal name of Thursday Island where the original specimens were collected. However, the species name was changed to *T. clypearis* in 1997[6] when our bees were shown to be identical with *T. clypearis* bees in New Guinea, Indonesia and the Philippines.

Estimated current distribution of Tetragonula clypearis

In Australia *T. clypearis* is found on Cape York Peninsula and the east coast of far north Queensland. In some colonies, the abdomens of *T. clypearis* workers look distinctly pale brown, especially if viewed with the light behind them. In other *Tetragonula* species, the abdomens of the workers are black.

These miniature bees have tiny nests and they often build in man made cavities. Nests are frequently seen in stone walls, wall cavities and hollow doors. We have also seen nests in flower pots, monuments and even in a tennis racquet cover.

Left: the brood comb of a T. clypearis nest, inside a wall cavity.

Most *T. clypearis* nests have a narrow entrance tunnel. The tunnel may be quite short or it may be up to 18 cm long, running along the outside of the log for some distance.

The brood cells of *T. clypearis* always open upwards but they are not connected together in any kind of hexagonal comb structure. Instead the cells are roughly arranged in diagonal rows.

Left: five T. clypearis bees peep out of their nest entrance tunnel. The nest is located inside the electrical conduit pipe below the tunnel. Right: a T. clypearis entrance tunnel that has been partially closed with a resin curtain at night.

An Overseas Relative — *Tetragonula biroi*

Overseas in New Guinea and in the Philippines there is a species that is closely related to our *T. carbonaria* bees. It is called *Tetragonula biroi* and was described by Heinrich Friese in 1898.

These bees look almost identical to *T. carbonaria* bees except they have paler colouring on their antennae. They also build nests that are similar to those of *T. carbonaria*.

Photographs posted in the Indo-Malayan Stingless Bees group on Facebook indicate that the brood comb structure of *T. biroi* sometimes resembles a stack of flat disks or it may be an irregular, partial spiral. Managed colonies can be kept in traditional hives made from a cluster of half coconut shells.

In the Philippines *T. biroi* is also kept in managed hive boxes by local beekeepers. Some *T. biroi* hives that were photographed in Los Baños by Evert Jan Robberts had built massive resinous entrance platforms on the outside of their boxes.

T. biroi is not found within Australia.

Right: a collection of T. biroi hives at the Bee Farm of the University of the Philippines Los Baños. Note the large entrance structures built by the bees. Below: a T. biroi hive being opened by Tetragonula expert, Alex Fajardo. Below right: close up of the T. biroi nest structures that resemble those of T. carbonaria in many aspects. Photographs by Evert Jan Robberts.

The Five Australian *Austroplebeia* Species

Currently five species of *Austroplebeia* are recognised in Australia: *A. cincta, A. essingtoni, A. australis, A. cassiae* and *A. magna*.[8] All of the *Austroplebeia* species, except *A. cincta,* build a simple cluster brood. So unfortunately nest structures are not as useful for distinguishing the *Austroplebeia* species as they are in the *Tetragonula*. Instead we use colour markings and certain features of the hair and body shape to identify the *Austroplebeia* species. The basic characteristics of the Australian species of *Austroplebeia* are as follows:

Species name & body length of worker	**Distribution**	**Colouring of workers**	**Remarks**	**More details**
Austroplebeia cincta 3.5 mm		Bright yellow bands on the face and thorax. A distinctive yellow patch underneath the wing on the side of the thorax.	Elongated nest entrance tunnels. Builds a unique brood comb with the new cells waxed into concentric shells.	Page 26.
Austroplebeia essingtoni 3.5 mm		Usually has broad cream markings on the face and thorax.	Usually nests in small hollow trees but may nest in wall cavities or crevices in cliffs.	Page 27.
Austroplebeia australis 4 mm		In eastern and western areas of the continent, the workers have two to four cream marks on the rear edge of the thorax. In central areas, the workers are more brightly marked with additional side bands on the thorax. The workers' abdomens may be black, ochre, red or even a glowing orange.	These bees vary in their colouring. However, our analyses showed that they are all the same species. Other old names for this species were: *A. percincta, A. cockerelli, A. ornata* and *A. websteri*. These four old names should no longer be used.	Pages 28–29.
Austroplebeia cassiae 4 mm		Dark. The rear edge of the thorax normally just has two dull cream marks or may be completely black.	The hair on the worker's face is much denser in *A. cassiae* than in *A. australis*. In recent years, these bees were often called *A. symei*.	Pages 29–30.
Austroplebeia magna 4 mm		Dark. The rear edge of the thorax may have two dull cream marks. Some workers have no cream marks at all on the face or thorax.	This is a new species we found in NT. The name refers to the broad segments on this bee's hind legs: 'magna' is a Latin word for 'large'.	Pages 30–31.

How to Identify the *Austroplebeia* Species Using their Colour Markings

The most obvious differences between the *Austroplebeia* bees are their colour markings. These markings can help us identify some of the *Austroplebeia* species; but for others the story is more complicated.

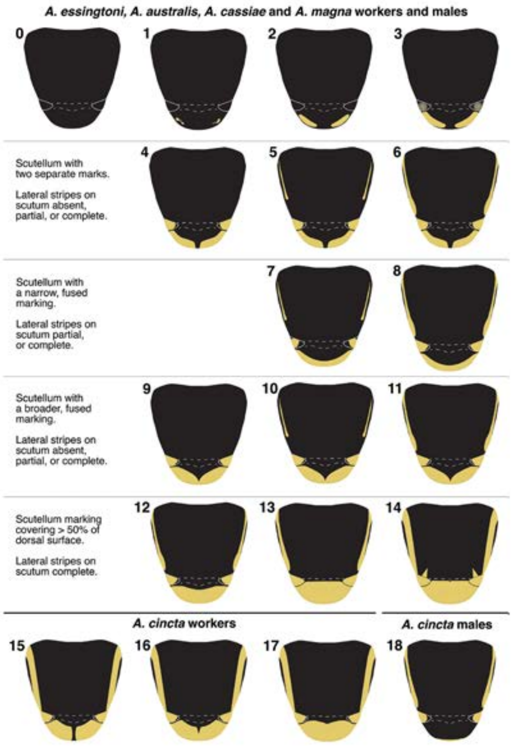
Over the years we have made detailed studies of 177 *Austroplebeia* nests from right across Australia. We examined up to 20 worker bees per nest, as well as males and queens where possible. Here is what we discovered about the colour markings of the *Austroplebeia*.[8]

Abdomen colour

Some *Austroplebeia* bees have quite bright orange abdomens. However, we do not think that the colour of the abdomen is important for species distinctions in the *Austroplebeia*. Abdomen colour varies a great deal. In some nests all the bees have orange abdomens, whilst in other nests some bees have orange abdomens and some have black abdomens. We have also seen abdomen colour in the same nest vary from year to year, perhaps following a change of queen bee.

Thorax and face colour markings

The cream or yellow markings on the thorax and face, however, are useful for species identification. We classified all the thorax markings that we saw into 18 colour grades. We coded these grades G0 through to G18. Diagrams of our thorax colour grades are shown in the chart on the right.



Austroplebeia thorax marking diagrams used in our analysis

The face markings are primarily helpful for identifying *A. cincta* and *A. essingtoni*. Diagrams of the major face marking grades are shown on the following pages. A layer of white hair covers the face in many *Austroplebeia* species. To view the face markings on these species, you need to tilt the bee's face back so you can see the markings under the hair.

A. australis colour morphs

In *A. australis*, workers in central areas of Australia have much brighter thorax colour markings than those in western and eastern areas (see map on page 28). Our studies indicate that, despite these colour differences, these bees are all the same species. We call the different colour varieties of this species 'Colour Morphs:

– *A. australis* Eastern Colour Morph – relatively dark bees found in NSW and QLD

– *A. australis* Central Colour Morph – relatively bright bees found in western QLD, NT and WA

– *A. australis* Western Colour Morph – relatively dark bees found in the Kimberley region of WA

Colour markings of the males

In the males, only *A. cincta* can be distinguished by using colour markings

In *A. essingtoni, A. australis, A. cassiae* and *A. magna*, all the males have similar markings, with bright broad bands on the sides and rear edge of the thorax (mostly G11 to G14, sometimes G5, G6 or G10). In *A. cincta* males, however, the rear edge of the thorax is black or it has just a faint yellow streak on its tip (G18).

Most males of A. essingtoni, A. australis, A. cassiae and A. magna have colour markings in the range G11 to G14		*In A. cincta males, the rear edge of the thorax is black or nearly so.*	

Colour markings of the workers

In the workers, some species can be identified by their colour markings but others cannot:

A. cincta These are the easiest workers to identify. They have long yellow or cream bands along the edges of their compound eyes, extending well above the antennae. They also have a distinctive colour patch (indicated with white arrow below) under the wing on the side of the thorax. These markings can distinguish this species in almost all cases. In addition they all have bright broad thorax markings (G15 to G17)

Distinctive colour markings seen on the face and on the side of the thorax (arrow) in workers of A. cincta

The typical thorax colour markings seen in workers of A. cincta

A. essingtoni Face markings are useful for identifying these workers. As shown below, most workers have much broader cream face markings on the face than in other species. *A. essingtoni* workers usually have bright thorax markings too. However, we did find some coastal populations that were substantially darker. For *A. essingtoni,* a combination of colour markings and size measurements (see page 23) works best.

The most common face marking seen in A. essingtoni workers was G6

In contrast, in A. australis, A. cassiae and A. magna workers, the face markings were usually G1 to G4.

Most A. essingtoni workers in our study had G13 thorax colour markings. A few particularly bright workers, that we saw in flower samples, had G14 markings.

In a few coastal areas, however, A. essingtoni workers were much darker and G8 was the most common colour marking grade.

A. australis, A. cassiae **and** ***A. magna*** In these species, colour markings are helpful in some cases (see charts on pages 19–21). However, it is often necessary to examine the bees' hair, bristles and leg shape too, to confirm your identification. This is explained in detail on pages 22–24.

The problem of variable markings

We were surprised to see how variable the worker bee colour markings were within the *Austroplebeia* species and even within individual nests. You can find workers with bright markings along with dull ones, and workers with a range of different abdomen colourings inside the same nest.

This variation can make it hard to identify individual *Austroplebeia* bees that have been caught on flowers. This is a particularly the case in Queensland, because the colour markings of the species there overlap so much (see page 19). For instance, a Queensland worker with G4 markings could belong to *A. cassiae* or to the Eastern or Central Colour Morphs of *A. australis*.

Nevertheless colour markings can be helpful for identifying the species of your *Austroplebeia* nest, if you can examine a sample of 10 to 20 workers caught from the nest. Try our three step identification guide!

Identification guide for an *Austroplebeia* nest

Step 1. Catch 10 to 20 workers from your nest

Flying foragers are best because their colour markings are fully developed. Many younger workers inside the nest are pale and have indistinct markings.

If you wish, you can catch a sample of workers and cool them down in your fridge (not your freezer!) for a few minutes until they go to sleep. Quickly examine and record the thorax markings of each bee (see Step 2). Finally let the bees warm up and they can fly away again.

Step 2. Work out the most common thorax colour grade in your bee sample

Look at the diagrams of the thorax colour grades shown on pages 19–21. Examine the markings of each bee using a dissecting microscope or a good 10x magnifying lens. Record all the bees' colour grades. Then work out which colour grade is the most common in your bee sample.

Step 3. Use the chart for your geographic area to make your identification

Turn to the chart that shows the *Austroplebeia* species in your state or territory:

– Queensland: page 19

– Northern Territory: page 20

– Western Australia: page 21

Note: we do not give a chart for New South Wales, because, at present, the only *Austroplebeia* species in that state is *A. australis*.

Examine the range of thorax colour grades shown for each species in your region:

– The two stars indicate the full range of colour markings that we saw for each species.

– The thickest part of the bar shows the most common markings that we saw in that species.

– Note that the workers within a single nest will not have the full range of colour markings shown for the species. There are colonies with darker and brighter colour markings within each species.

Find your nest's most common colour grade on the bottom row and see which species have workers with that marking:

You will notice that a particular colour grade may be found in two or three different species. In this case, the species of your nest is probably the one in which that grade is the most common. However, it is important to remember that your nest could belong to one of the other species. This is where we need to use other characters to confirm the identification, such as hair thickness and leg shape. These extra characters are explained on pages 22–25.

Example 1.
If you live in Queensland and the most common colour grade in your nest is G0, look at the chart on page 19. In Queensland, colour grade G0 is found in *A. cassiae* and also in the Eastern Colour Morph of *A. australis*. However, G0 is much more commonly found in *A. cassiae*; so that is the most likely identification for your nest. Nevertheless your nest could also belong to the Eastern Colour Morph of *A. australis*.

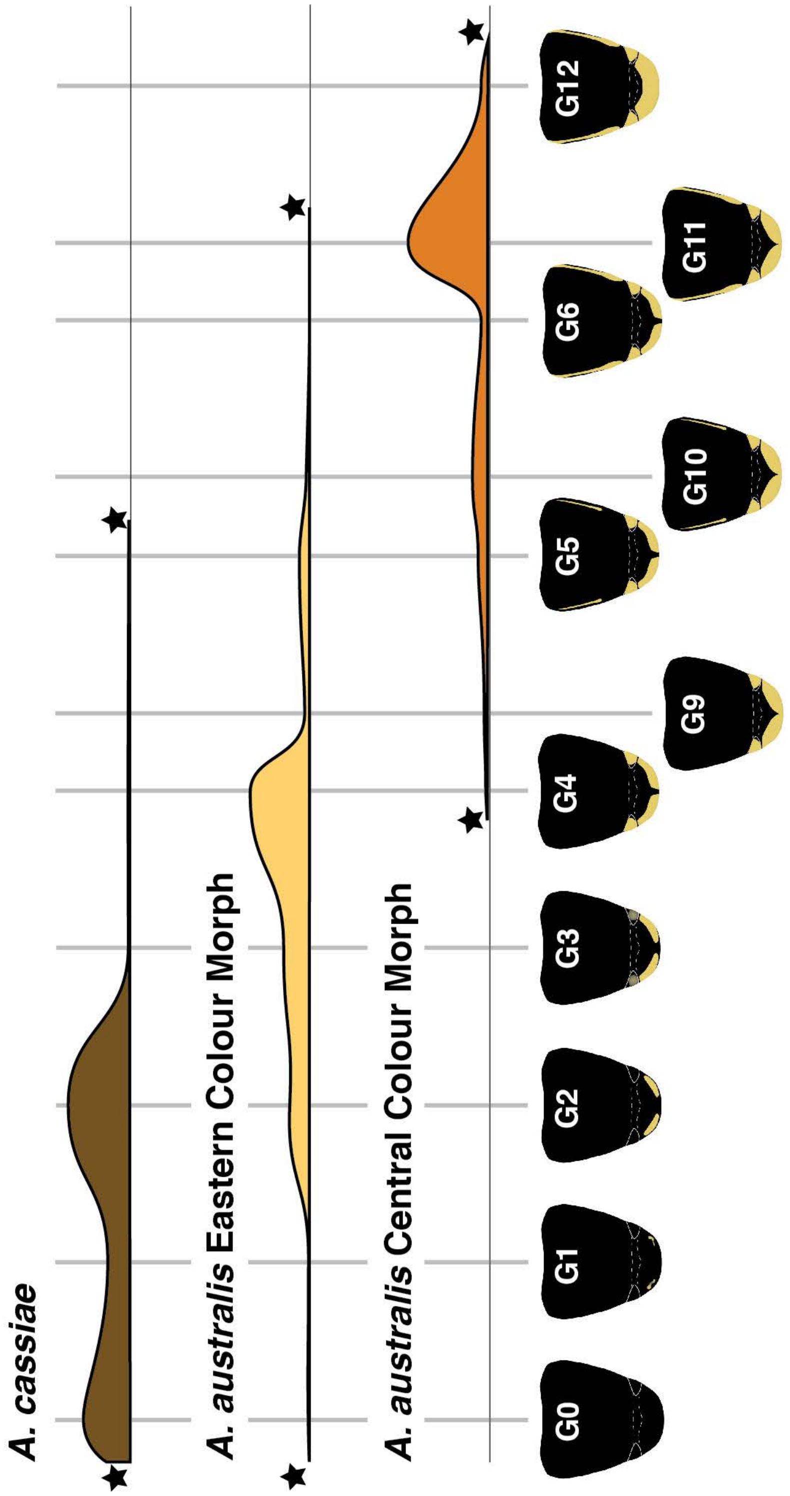
Example 2.
If you live in Queensland and the most common colour grade in your nest is G2, look at the chart on page 19. In Queensland, colour grade G2 is found in *A. cassiae* and the Eastern Colour Morph of *A. australis*. G2 is more commonly found in *A. cassiae*; so that is the more likely identification for your nest. However, G2 is also fairly common in the Eastern Colour Morph of *A. australis*. (In fact, in some *A. australis* nests, G2 is the most common colour grade!) In this case you would need to check the other characters on pages 22–25 to make a final identification.

Species of *Austroplebeia* in Queensland

Austroplebeia cincta: Small populations of *A. cincta* occur in the Daintree and Atherton Tablelands areas of Queensland. We have not included *A. cincta* in our colour chart below because this species is easy to identify by the distinctive colour markings on its face and the side of its thorax. See pages 17 and 23 for further details and diagrams.

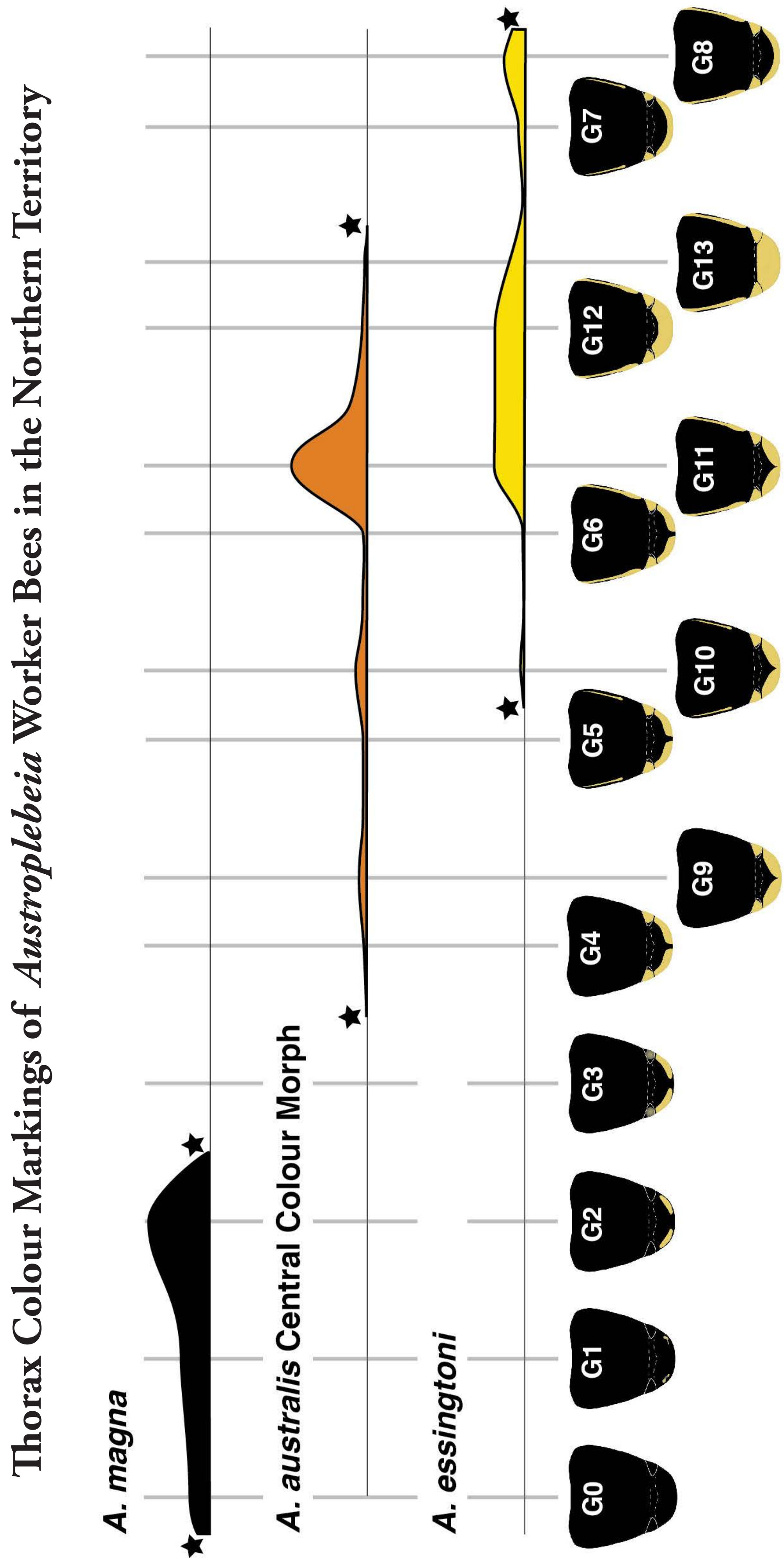
New South Wales: We have not given a separate colour chart for New South Wales because the only species that currently occurs in that state is *A. australis*.

Thorax Colour Markings of *Austroplebeia* Worker Bees in Queensland



The distribution of *A. magna* extends slightly into Queensland, near the border with the Northern Territory on the shores of the Gulf of Carpentaria. The colour markings of *A. magna* are given on the Northern Territory colour chart.

Species of *Austroplebeia* in Northern Territory

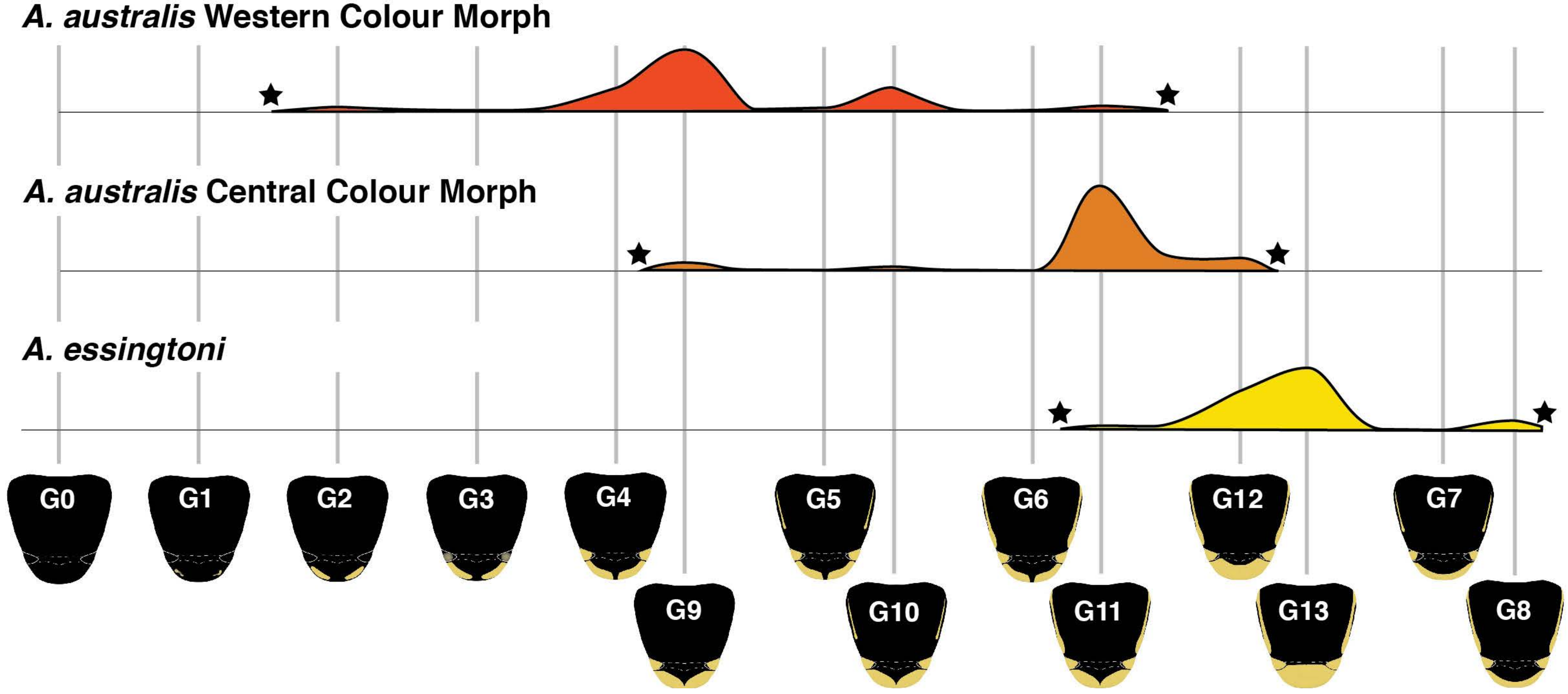


G7 and G8 are two relatively dark colour marking patterns that we have only observed in *A. essingtoni*.

The populations of *A. essingtoni* that we observed in the Northern Territory tended to have darker colour markings than those we observed in Western Australia.

Species of *Austroplebeia* in Western Australia

Thorax Colour Markings of *Austroplebeia* Worker Bees in Western Australia



G7 and G8 are two relatively dark colour markings that we have only observed in *A. essingtoni*.

We have only found populations of *A. australis* Western Colour Morph in far northern areas of the Kimberley.

The distribution of *A. magna* may extend slightly into Western Australia in the Kimberley region. The colour markings of *A. magna* are shown on the colour chart for the Northern Territory.

Further Identification Tips for *Austroplebeia*
— for the technically minded

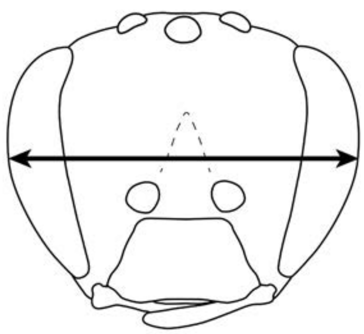
On pages 16–21, we explored how to use colour markings to identify the *Austroplebeia* species. In some cases, the species could be recognised by their colour markings; but in other cases the identification was unclear.

In our *Austroplebeia* revision research, we found that certain characteristics of the bees' hair and bristles (thickness and colour) and body shape (head width and hind basitarsus width) provided valuable additional information for species identification. The following is a more detailed method for identifying the *Austroplebeia* species using measurements and hair patterns. The full version of this key is given in our 2015 revision paper.[8]

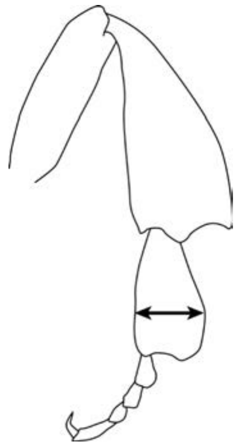
For instructions on how to set up a microscope to take precise measurements of bees, see Appendix 1 on page 34.

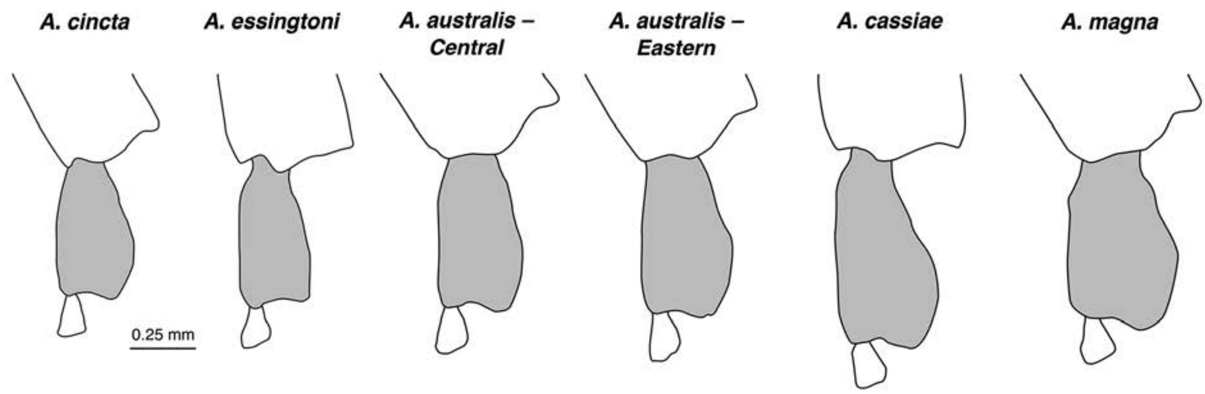
Measurements of a bee's overall body length are not very helpful. A bee can expand and contract its abdomen, causing a substantial difference in the length of its body. Instead we use the following two measurements of the worker bees in this method:

Head width



Hind basitarsus width





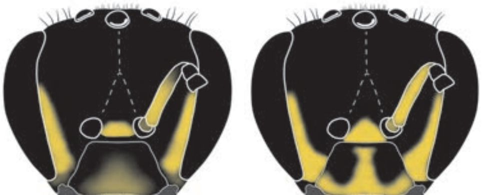
We found that the average width of the worker bee's hind basitarsus segment varies between the different species, as shown in this diagram.

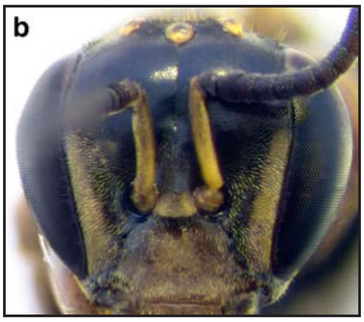
The size ranges given in the following tables are based on measurements we did on hundreds of worker bees sampled from nests across Australia over the past two decades. We found that workers in the same nest may vary in size. So you need to measure several workers from the same nest and average their scores to get the most accurate result. Even though many of the size ranges in these tables overlap, these measurements may provide a more reliable species identification than colour characteristics in the *Austroplebeia*.

Step 1. Separate *A. cincta* from remaining *Austroplebeia* species

A. cincta workers can be readily distinguished from those of other species by their colour markings and hair. They also build a distinctive brood structure.

	Distribution	Marking on side of thorax under wing	Face markings of workers	Hair on face and side of thorax of workers	Larval cells in brood
A. cincta	New Guinea and northeastern QLD	Present in workers, as shown below left (arrow)	With long stripes beside the eyes, as shown below	Surface looks shiny because the hairs are very fine, as shown below	Waxed tightly together into single layer, concentric shells (see page 26)
***A. essingtoni, A. australis, A. cassiae & A. magna...* Go to step 2**	WA, NT, QLD and NSW	Absent in workers, except in the brightest *A. essingtoni*	See diagrams in Step 2	Surface with numerous whitish feathery hairs (see photos in Step 4)	Loosely connected into a cluster structure (see pages 27 and 29–31)





Above left: A. cincta workers have a distinctive cream patch (arrow) under their wing. (The males lack this marking.) Above centre: the range of A. cincta face markings that we observed in workers in Australia and New Guinea. They all have long narrow stripes along the edge of their compound eyes, extending well above the level of the antennae. Above right: the face of an A. cincta worker appears shiny because the hairs on the face are very fine. Other species of Austroplebeia have a noticeable coating of whitish feathery hair on the worker face (see photographs on page 24).

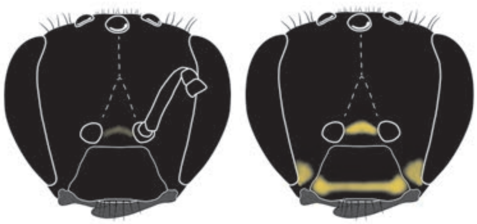
Note: Professor Michener suggested in 1990 that a tiny wing vein (called the 'vestige of the first transverse cubital vein') was primarily found in *A. cincta*.[3] However, our studies have shown that this characteristic may be found in all the *Austroplebeia* species. So unfortunately it is not helpful for distinguishing *A. cincta* from other *Austroplebeia* species.

Step 2. Separate *A. essingtoni* from *A. australis, A. cassiae* and *A. magna*

In most areas, *A. essingtoni* is markedly smaller than *A. australis, A. cassiae* and *A. magna*. The workers usually also have more extensive face markings.

	Distribution	Face markings of workers	Head width of workers
A. essingtoni	WA and NT	Usually as shown below left	1.44–1.67 mm
***A. australis, A. cassiae & A. magna...* Go to step 3**	WA, NT, QLD and NSW	Usually as shown below right	1.59–2.01 mm (usually greater than 1.65 mm)

Left and centre: the most common face markings in A. essingtoni workers. Right: even in the most brightly A. essingtoni workers, the face markings only extend to the level of the antennae.

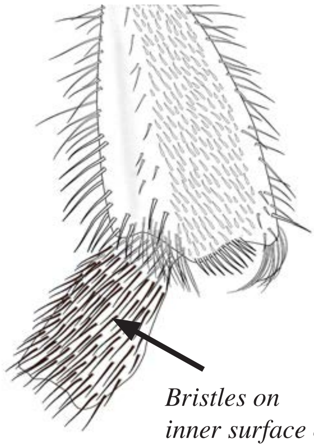


Range of common face markings in A. australis, A. cassiae and A. magna workers

Step 3. Separate *A. australis* from *A. cassiae* and *A. magna*

A. australis workers generally have brighter colour markings and paler hind basitarsus bristles than workers of the other species. Their hind basitarsus is usually also narrower.

	Distribution	**Thorax markings of workers (see diagrams below)**	**Colour of bristles inside hind basitarsus in mature workers (see diagram below)**	**Width of worker hind basitarsus (see page 22)**
A. australis	WA, NT, QLD and NSW	Usually G2–G11	Gold or pale gold	0.29–0.40 mm
***A. cassiae* & *A. magna*... Go to step 4**	WA, NT, QLD and NSW	Usually G0–G2	Brown or slightly brown	0.35–0.47 mm

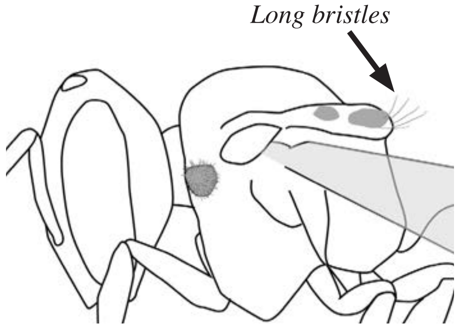


Above: the range of common worker thorax colour grades seen in A. australis (grades G2 to G11), and in A. cassiae and A. magna (grades G0 to G2).
Right: diagram showing the coarse bristles inside the basitarsus segment of the hind leg of workers. In A. australis these bristles are golden or pale gold; whilst in A. cassiae and A. magna they are brown or slightly brown.

Step 4. Separate *A. cassiae* from *A. magna*

A. magna workers have the broadest hind basitarsus of the *Austroplebeia* species. These two species also differ slightly in the worker hair patterns and colour, and have different distributions.

	Distribution	**Feathery hair on face of workers (see photographs below)**	**Colour of bristles on rear edge of thorax in mature workers (see diagram below)**	**Width of worker hind basitarsus (see page 22)**
A. cassiae	QLD	Usually dense	Usually coarse and opaque brown	0.35–0.44 mm
A. magna	NT and far north-western QLD	Usually more sparse	Finer with slight brown tinge or pale	0.39–0.47 mm



Above left: the faces of worker bees of A. cassiae (on left) and A. magna (on right) showing that the whitish feathery hair on the lower part of the face is much thicker in A. cassiae than it is in A. magna.
Above right: the rear edge of the worker's thorax has many long bristles (see arrow). These bristles are darker and coarser in A. cassiae than they are in A. magna. Examine them using a microscope at high magnification, with the bristles viewed against a white or pale background (such as one of the cream markings in this area).

Additional Information about *Austroplebeia* Males and Queens

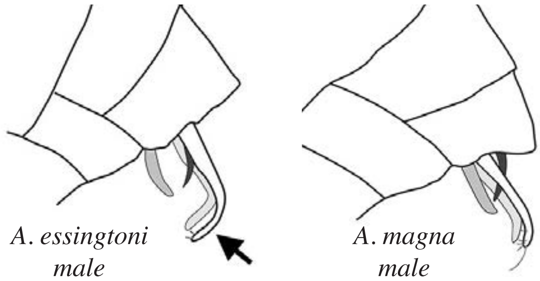
A. cincta

In *A. cincta* males, the rear edge of the thorax is virtually black (see page 16). In all the other species, the males have broad colour bands in this area.

A. essingtoni

In *A. essingtoni* males, the hardened genitalia have distinct bends near the tip, as shown in the diagram on the left (arrow). This structure can be seen in many specimens protruding from the end of the abdomen.

In *A. cincta, A. australis, A. cassiae* and *A. magna* males, the tips of the genitalia are gently curved, as shown in the diagram on the right.



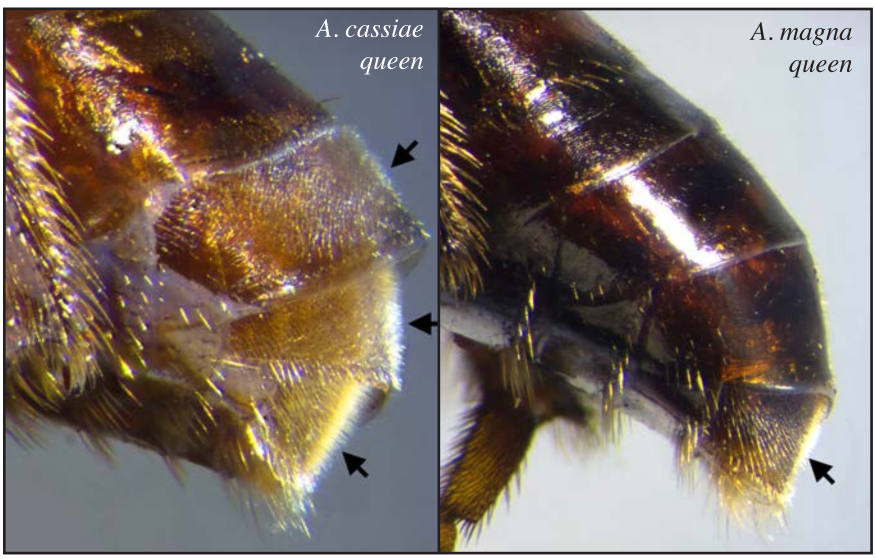
(Diagrams show side view of end of abdomen.)

A. australis, A. cassiae and *A. magna*

Males of these three species may be distinguished by examining the hairs on the side of the thorax with a high quality microscope. The shape of the feathery hairs in these three species varies, as shown in the following photographs taken by Dr Ken Walker, Museum Victoria.

Mature queens of these three species may be distinguished by looking at the final three segments of the abdomen, as shown in these photographs.

In queens of *A. cassiae*, the final three segments are covered with dense short hair. In *A. australis* and *A. magna*, only the final segment is covered with dense hair; the other two segments are shiny and almost hairless.

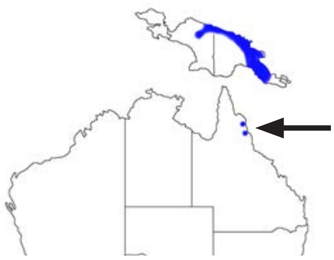


Queen abdomen segments that are covered with dense short hair (indicated with the arrows)

More Details About the Five Known Species of *Austroplebeia* in Australia

Austroplebeia cincta

The Hungarian field entomologist, Lajos Biró, collected the first specimen of *A. cincta* near Friedrich Wilhelmshafen (now known as Madang, Papua New Guinea) in 1896. It was sent to a museum in Budapest, Hungary. The museum curator, Alexander Mocsáry, described this species in Latin in 1898.



Estimated current distribution of Austroplebeia cincta

A. cincta is mainly found in New Guinea. From an extensive search for museum specimens and old journal records, we found that *A. cincta* occurs in coastal and mountainous areas of Papua New Guinea, as well as in the Indonesian province of Papua. In 2010 we also identified *A. cincta* in two small areas (marked by the arrow) of the Daintree and Atherton Tablelands in North Queensland. These are all areas with high annual rainfall.

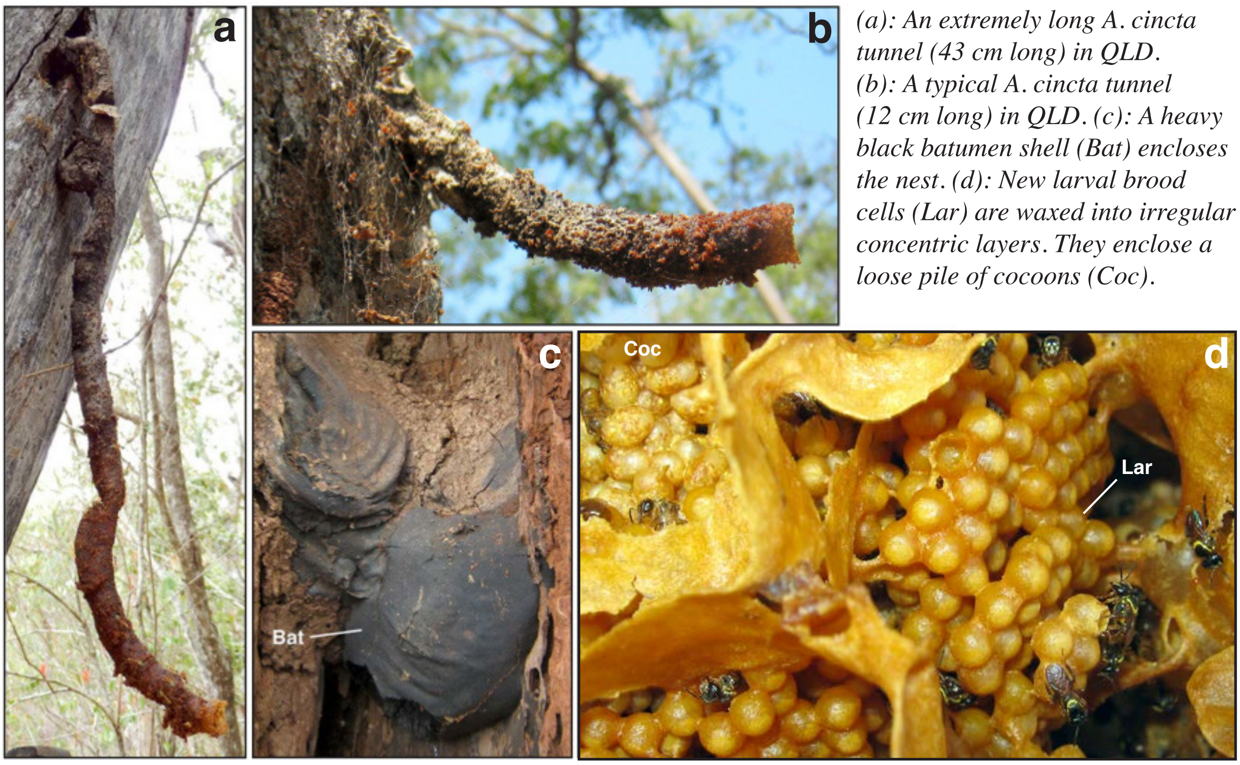
Another *A. cincta* population in 'N. Australia' was mentioned decades ago in *The Social Behaviour of the Bees*[11] and the *Zoological Catalogue of Australia*[12]. This record was based on some Western Australian bees that had been collected in by Eric Mjöberg in 1910–1911. However, our research has shown that these bees were not *A. cincta* – they actually were *A. essingtoni*. So, to our knowledge, the only Australian records of *A. cincta* are the two recently identified populations in northeastern Queensland.

These tiny bees have bright yellow bands on their face and thorax ('cincta' is a Latin word for 'bordered'). Workers also have a distinctive yellow patch underneath the wing on the side of the thorax. Unlike all other *Austroplebeia* species, the males of *A. cincta* that we sampled in Queensland were darker than the workers, lacking some of the thorax markings seen in the workers. No male or queen specimens from New Guinea have been found so far in museum collections.

A. cincta bees are remarkable tunnel builders, as we observed during our 2012 safari to north Queensland. Flying foragers are harassed by aggressive green ants in these areas. So the bees build elongated nest entrance tunnels. They keep extending the tunnel by adding sticky resin to its tip. Finally the tunnel collapses under its own weight and the bees immediately get to work on constructing a new tunnel. One exceptional tunnel we saw was 43 cm long!

Nests are built in cavities inside relatively large trees (22–80 cm diameter at nest level). The bees create a suitably-sized space for the nest by closing off part of the cavity with heavy black batumen walls.

This is the only *Austroplebeia* species that does not build its brood in a cluster structure. Instead the larval cells are waxed together into small single layer combs. These combs interconnect to form irregular concentric shells that enclose the cocoons. Similar brood structures were observed in New Guinea nests of *A. cincta* by Professor Michener.[13]



(a): An extremely long A. cincta tunnel (43 cm long) in QLD. (b): A typical A. cincta tunnel (12 cm long) in QLD. (c): A heavy black batumen shell (Bat) encloses the nest. (d): New larval brood cells (Lar) are waxed into irregular concentric layers. They enclose a loose pile of cocoons (Coc).

Austroplebeia essingtoni

Estimated current distribution of Austroplebeia essingtoni

The earliest specimens of this species were collected from a tiny British outpost at Port Essington on the north coast of Arnhem Land, Northern Territory, in about 1840. The bees were shipped to England and were purchased by the Natural History Museum in London in 1842. Professor TDA Cockerell, an American bee expert, examined them there six decades later. He described them as a new species in 1905, naming them after place where they were collected.

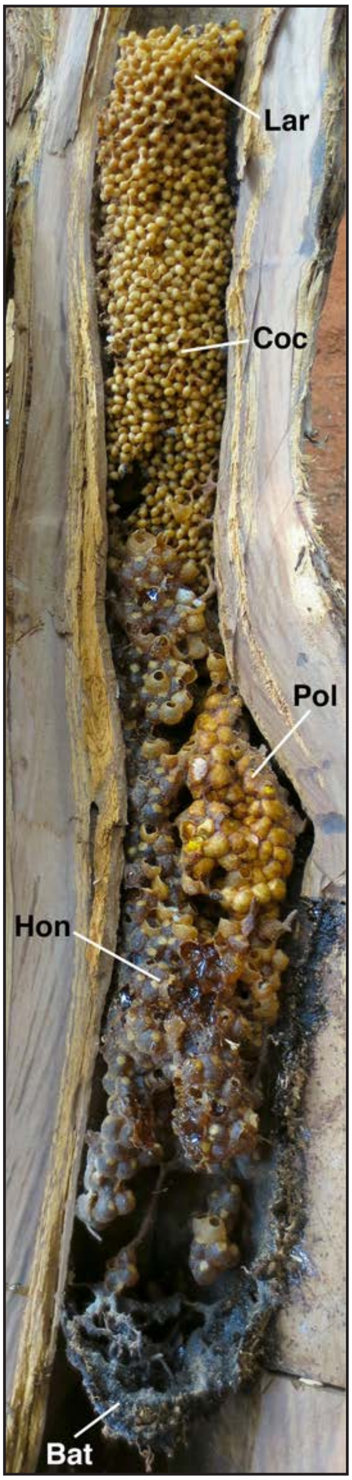
In 1987 we trekked through Arnhem Land to Port Essington to look for this species. After a week's search, we finally discovered two nests of *A. essingtoni* in spindly dead trees. On an earlier native bee safari through Western Australia, we had found other nests of this beautiful bee. The surveys we recently completed for our revision paper(8) revealed that this species is widely distributed in northern areas of Western Australia and Northern Territory: in the Hamersley Ranges, The Kimberley and in Arnhem Land. They can survive in some quite arid areas with annual rainfalls down to 300 mm.

Workers of this species are reasonably easy to identify. *A. cincta* (not found in Western Australia or Northern Territory) is the only other *Austroplebeia* with similar colouring and size. *A. essingtoni* workers usually have broad cream markings on the lower face and distinct cream bands on the side and rear of the thorax. However, their colouring varies. In some coastal areas, the workers are much darker (as shown on page 17). Most workers are noticeably smaller than those of the other *Austroplebeia* species in their range.

A. essingtoni males all have unusual bent tips on their genitalia. These tips can often be seen in dried bees protruding from the end of their abdomen (as shown in the drawing on page 25).

Nests are normally built inside small to medium hollow trees (9–30 cm diameter at nest level). However, this is the only *Austroplebeia* species, which, according to our studies, sometimes also nests in wall cavities or in cliff crevices. The bees usually build a short nest entrance tunnel.

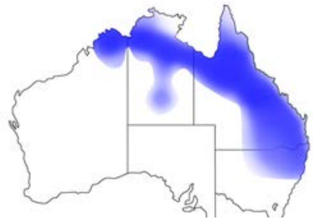
In the brood, the spherical waxy cells (that contain the larvae) are loosely connected together into an irregular structure called a 'cluster'. In a cluster brood, the newly built cells may open upwards, downwards or horizontally, but they face towards the outside of the brood. The honey and pollen is stored in spherical or oval shaped pots with thin walls.



Left: a nest of A. essingtoni inside a hollow log. The cluster brood consists of waxy cells (Lar) containing the larvae and silken cocoons (Coc) containing the pupae. Below the brood, are pots containing pollen (Pol) and honey (Hon). A hard batumen layer (Bat) seals off the bottom of the nest. Photograph by Tim Heard.
Below: the entrance tunnel of a nest of A. essingtoni which is inside a crevice in a rocky cliff. Photograph by Aung Si.

Austroplebeia australis

The earliest specimens of *A. australis* were collected in Central Australia in about 1891. The bees were labelled with the name of Ferdinand von Müller, the Government botanist of Victoria. However, it is thought that the missionary Adolf Hermann Kempe from Hermannsburg Mission, west of Alice Springs, NT, actually arranged the collection of these bees with the help of local Aboriginal people.



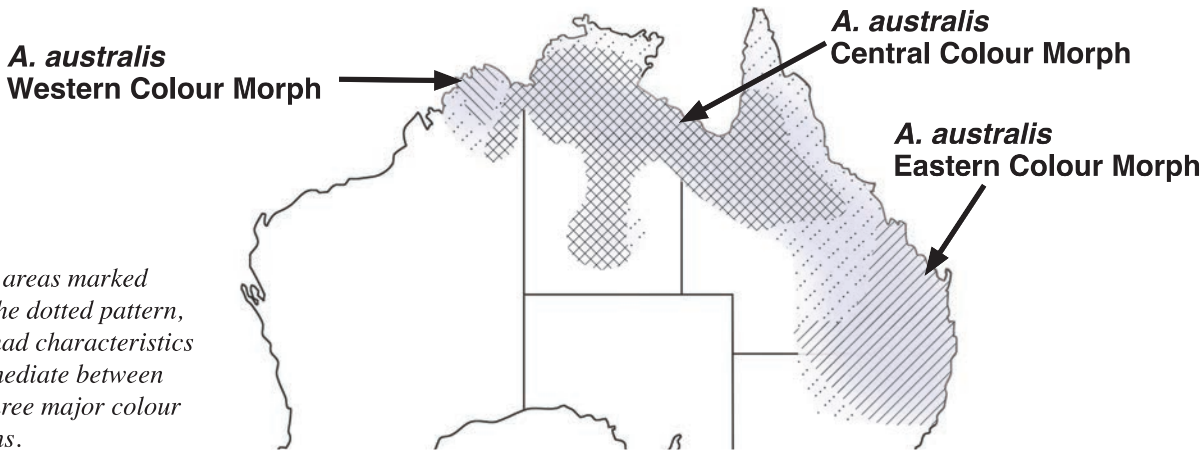
Estimated current distribution of Austroplebeia australis

Many of the bees were sent to museums in Germany. Heinrich Friese, a German bee expert, examined numerous specimens then held by the Museum of Stuttgart, when he described this species in 1898. The species name refers to the bees' origin in Australia ('australis' is Latin for 'of the south').

A. australis is the *Austroplebeia* species that is most frequently kept in hive boxes in Queensland. However, our studies show that this is actually a very widespread species, also found in New South Wales, Northern Territory and Western Australia. This is the *Austroplebeia* species that extends furthest south in Australia. It has been found as far south as Dungog and Wootton in New South Wales (latitude 32.4^{o} S). In central parts of Australia, *A. australis* can cope with quite arid conditions, with annual rainfalls down to 300 mm.

A. australis bees in eastern, central and western parts of Australia differ markedly in their colouring. Nevertheless they all belong to the same species, because their characteristics change gradually from one local population to the next, right across the continent.

The workers from central areas are the most brightly marked, with cream bands on the sides of the thorax. The workers in the eastern and western areas are darker and do not have side bands on the thorax. In our revision paper,[(8)] we called these three different populations 'colour morphs' and we analysed in detail the distributions and characteristics of bees in the Eastern, Central and Western Colour Morphs of *A. australis*.



In the areas marked with the dotted pattern, bees had characteristics intermediate between the three major colour morphs.

Some Central Colour Morph workers, especially in western Queensland, have bright orange or red abdomens. However, abdomen colour is very variable, even within a nest. Although this characteristic is extremely eye-catching, we think that it is just a colour variant within the *A. australis* species.

A. australis males are all brightly marked, with cream bands on the face, thorax and legs. The tips of their genitalia curve gently and can easily be distinguished from those of *A. essingtoni* males (see page 25).

These attractive bees were popular with early collectors and four additional species names were published between 1929 and 1932 for these bees. However, our analyses show that they are all the same as the *A. australis* species:

— *A. percincta* (from Hermannsburg, Northern Territory),
— *A. cockerelli* (from Borroloola, Northern Territory),
— *A. ornata* (from Cape York, Queensland), and
— *A. websteri* (from Wyndham, Western Australia)

These four species names were published three decades after the publication of the *A. australis* name. The rules for naming animals say that we must use the oldest name that has been published for a species. So these four names should no longer be used and we need to call all of these bees *Austroplebeia australis*.

The nests of *A. australis* are similar to those of *A. essingtoni*. Nests were found in trees of various sizes (8–90 cm diameter at nest level). In the Eastern Colour Morph, relatively large nests were built in broader tree cavities (average 10 cm diameter), and most colonies had a short nest entrance tunnel. In the Central Colour Morph, smaller nests were built in narrower tree cavities (average 5 cm diameter), and most colonies had no tunnel at all, with at most a smear of resin around the entrance.

A. australis builds a cluster brood comb and food storage pots similar to those of *A. essingtoni*. However, the storage pots are sometimes heavily reinforced with many waxy deposits, making them appear pitted.

Top left: a translucent fresh tunnel built by an A. australis colony in Queensland. Photograph by Megan Halcroft.
Above left: An A. australis colony without an entrance tunnel in Queensland. Photograph by Megan Halcroft.
Above right: The cluster brood of an A. australis colony in Central Australia.

Austroplebeia cassiae

Estimated current distribution of Austroplebeia cassiae

In the late 1800s, two brothers, Rowland and Gilbert Turner, collected bees in the Mackay area of Queensland for 18 years, resulting in the discovery of 97 new species. They collected the first *A. cassiae* specimen at Mackay in 1899.

The specimen was sent, with many others, to the Natural History Museum in London. Professor TDA Cockerell described this species in 1910. He named it after the *Cassia* flowers from which the type specimen had been collected.

This species is common on Cape York and in coastal areas of eastern Queensland. However, we have only found natural colonies in relatively high rainfall parts of Queensland (annual rainfall above 600 mm). This is the second most common *Austroplebeia* species that is kept in managed hives.

In recent years, beekeepers have been calling this species *A. symei* — a species name that was published in 1932. However, our analyses showed that *A. symei* is the same as the older species, *A. cassiae*. The rules for naming animals say that the older name must be used, so we now should call these bees *Austroplebeia cassiae*.

A. cassiae workers are generally dark but those in our study always had at least a small, dull, crescent-shaped marking between the antennae on the face, hidden under the white hair. The rear edge of the thorax normally has just two small, dull markings or it is completely black (more details on pages 19 and 24). As in *A. australis*, the males are brightly marked and have gently curving tips on their genitalia (see page 25).

Professor CD Michener once suggested that *A. cassiae* was the same as the species *A. australis,* on the basis of the colouring of their mandibles.[13] Our analyses show, however, that these are two separate species. Mandible colour is variable in both species.

The thorax colour markings of *A. cassiae* workers in some nests can be identical to those of *A. australis* workers. Unfortunately this means that it sometimes it is not possible to distinguish *A. cassiae* and *A. australis* on the basis of colour markings alone. However, there are three other characteristics which can help to distinguish *A. cassiae* and *A. australis*. *A. cassiae* workers have denser white hair on the lower face, broader hind basitarsus segments and darker hind basitarsus bristles than *A. australis* workers do. More details are given on page 24 (the face hair of *A. australis* is similar to that shown for *A. magna*).

In 1987 we came across a property in central Queensland that was rich in both *A. cassiae* and *A. australis*. The property owner, Ernie Adams, was an expert at finding bees. With Ernie's help, we documented 11 nests of *A. cassiae* and 12 nests of *A. australis* on his property. Even though the nests were all close together in the same paddocks, they maintained their distinct characteristics, showing that they are separate species. Later Megan Halcroft confirmed the species identity of nine of these colonies with her DNA analyses.[7]

We found nests of this species in trees of various sizes (9–37 cm diameter at nest level). *A. cassiae* builds relatively large nests that usually have a short entrance tunnel. However, as in other *Austroplebeia* species, the bees may build elongated entrance tunnels if they are being harassed by green ants. The brood structure and storage pots are similar to those of *A. australis*.

Top left: the thickened, older entrance tunnel of an A. cassiae nest. Above left: a 160 mm long entrance tunnel built by an A. cassiae colony that was being harassed by green ants. The long tunnel helps keep the ants out of the nest. Above right: The cluster brood of a colony of A. cassiae that was being kept in a hive box.

Austroplebeia magna

During our 1987 native bee safari Arnhem Land, Northern Territory, we discovered nests of a large dark *Austroplebeia* bee. Although these dark bees were not the goal of our safari, we still systematically studied them and carefully stored away some samples. This was most fortunate, because our recent analyses revealed that they were actually a brand new species!

During this safari, we also found other nests of these dark bees further south, near Katherine, Northern Territory. We chose worker and male bees from one of these nests for the type specimens. We described this new species in our 2015 *Austroplebeia* revision paper.[8]

A. magna is mainly found in northern parts of Northern Territory but we have traced its range along the southern shores of the Gulf of Carpentaria and a short distance into western Queensland. Its distribution was in areas with an annual rainfall of more than 600 mm.

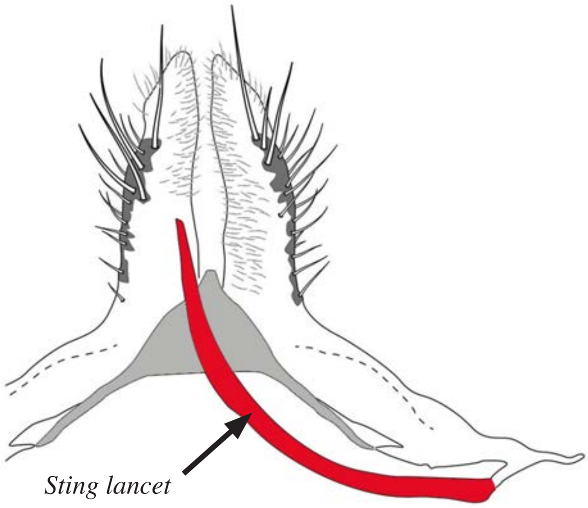
Estimated current distribution of Austroplebeia magna

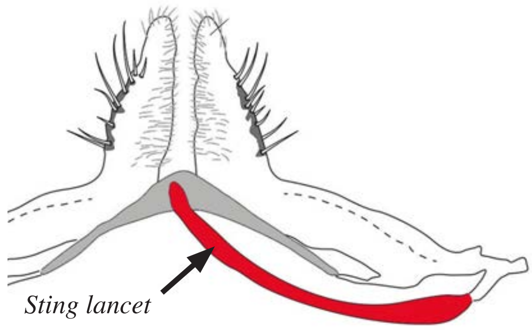
Many *A. magna* workers have two dull cream marks on the rear edge of their thorax (see page 20). However, some northern workers were the only *Austroplebeia* bees we found in our study that had no cream markings at all on their face or thorax.

A. magna males are brightly marked, with cream bands on the face, thorax and legs. As in *A. australis* and *A. cassiae*, the tips of their genitalia are gently curved (see page 25).

A. magna workers look quite similar to those of the Queensland species, *A. cassiae*, but a surprising discovery showed us that *A. magna* and *A. cassiae* were separate species. Queens usually show relatively few differences between species. However, we found a very clear-cut difference between the mature queens of these two species, in the hair patterns on their upper abdomens. In *A. cassiae*, the final three segments of the queen's abdomen were covered with short dense hair. However, in *A. magna*, only the final segment had short dense hair; the other two segments were shiny and almost hairless (see photographs on page 25).

Even though *Austroplebeia* bees are 'stingless', a minute remnant of the sting structure still exists inside the abdomen of the worker bees. The entire structure, shown below, is only half a millimetre wide and has to be examined under a powerful microscope. Measurements of minute features of the sting rudiments confirmed the identities of the *Austroplebeia* species in our research.





The sting rudiments of A. magna (on left) and A. essingtoni (above) shown at the same magnification. The sting lancet of A. magna is much longer than that of A. essingtoni or the other Austroplebeia species.

We found that *A. magna* workers had the longest sting lancets of all the *Austroplebeia* species. Some workers also had the broadest basitarsus segments on their hind legs that we saw in our study (see page 22). For these reasons we chose the name *Austroplebeia magna* for our new species ('magna' is the Latin word for 'large').

All nests that we studied of *A. magna* were in small to medium sized hollow trees (10–24 cm diameter at nest level). The bees usually built a short nest entrance tunnel. The brood structure and storage pots were similar to those of *A. australis*.

Above left: the entrance tunnel of an A. magna nest. Above right: the cluster brood of an A. magna nest that is inside a narrow tree cavity. Both photographs by Aung Si.

For more *Austroplebeia* information and photographs,
download your free copy of *Aussie Bee Online* Article 25,

Meet the *Austroplebeia* Species.

Visit: **www.aussiebee.com.au/abol-current.html**

Conclusion

We have learned a great deal about the differences between the species of the Australian stingless bees in recent years. However, due to their tiny size, the species and castes are not easy to recognise. Nevertheless with practice you will get to know the bees in your area well and in the process you will surely learn much about their habits.

The secret lives of our Australian stingless bees are fascinating to study. How many other people can claim they need a microscope to see their pets!

Anne Dollin studying the Austroplebeia stingless bees

Glossary

Abdomen – A bee's body has three sections, the head, the thorax and the abdomen. The abdomen is the third or tail section (see diagram on page 2).

Basitarsus – A broad segment of the bee's leg, just below the tibia (see diagram on page 2).

Brood – The structure in the centre of the nest where the eggs are laid. It consists of hundreds of cells, about 3 mm wide. The cells containing the larvae are made of wax and resin; whilst the cocoons containing the pupae are made of silk. See our ebook, *Behaviour of Australian Stingless Bees*, for more information — details below.

Castes – Different kinds of bees with distinct functions within a colony of bees. There are three castes within a colony of stingless bees: the queen, the workers and the males (or drones).

Cluster – A group of brood cells that are in a loose irregular arrangement, not in combs.

Colour morph – A major population within a species. The bees in each colour morph have similar colour markings.

Corbicula (or pollen basket) – A broad polished area surrounded by hairs on the outside of the tibia section of the hind leg (see diagram on page 2). Stingless bees carry pollen and resin on the corbicula.

Keirotrichia – A band of short blunt bristles on the inside of the tibia section of the hind leg (see diagrams on page 7). It appears to be used for cleaning the wings.

Mandibles - The jaws of the bee, used for handling nest materials and for defense (see diagram on page 2).

Sting rudiments – A minute remnant of the sting structure that still exists inside the tip of the abdomen of a stingless bee (see diagrams on page 31).

Thorax – A bee's body has three sections, the head, the thorax and the abdomen. The thorax is the second section, corresponding with the chest (see diagram on page 2).

Tibia – A long segment near the middle of the bee's leg (see diagram on page 2). The hind tibia carries the corbicula on the outside and the keirotrichia on the inside.

Queen – In a social bee nest, there are three kinds of bees: a queen, workers and males. The queen is the fertile female bee that normally lays all the eggs in a nest.

Worker – In a social bee nest, there are three kinds of bees: a queen, workers and males. The workers are the sterile female bees that do most of the work in the nest.

Further Reading

Other eBooks in the *Native Bees of Australia Series*:

— *Introduction to Australian Native Bees*
— *Nests of Australian Stingless Bees*
— *Behaviour of Australian Stingless Bees*
— *Keeping Australian Stingless Bees in a Log or Box*
— *Boxing and Splitting Hives: A Complete Do-It-Yourself Guide*

The above ebooks are available from the Aussie Bee website: **www.aussiebee.com.au/abshop.html**

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Finally, a special thank you goes to the co-authors who worked with us on our stingless bee revision papers, Dr Claus Rasmussen and the late Professor Shôichi Sakagami. We greatly appreciated the expertise that they generously provided in our studies of the Australian stingless bee species.

Some of the images reproduced on pages 7, 16, 17, 19–27 and 29–31 of this booklet were first published in Zootaxa, 4047: 1–73.

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Appendix 1
How to Measure Bees Using a Microscope

How to take measurements of bees for species identification

To confirm the identity of many of our Australian stingless bee species, it is useful to measure some structures of the bees themselves, very precisely, to the nearest 0.01mm. Measurements of the lengths of the bees' wings and the widths of their heads and leg segments can help you make much more accurate species identifications.

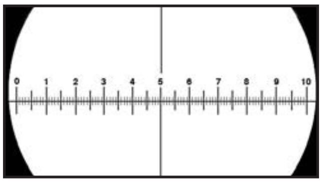
Equipment required

To make these measurements, you need the following equipment:

– a dissecting microscope with a total magnification of 15x to 40x (combining the zoom and eyepiece magnifications).

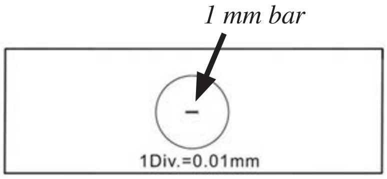
– an eyepiece graticule (available from ebay). You can either buy a glass graticule to insert inside the eyepiece of your microscope or a complete eyepiece with a graticule already installed.

– a stage micrometer calibration slide (available from ebay). This slide, with a 1mm long bar etched precisely onto it, is used to calibrate the measurements made with the eyepiece graticule.



Left: this is how the eyepiece graticule should appear, when looking through the microscope eyepiece.

Right: the stage micrometer slide that is used to calibrate the measurements.



How to calibrate your microscope

Step 1. Place the stage micrometer slide onto the stage of the dissecting microscope. Focus on the etched 1mm bar, as sharply as possible, whilst looking through the eyepiece containing the eyepiece graticule.

Step 2. Count how many divisions of the eyepiece graticule are equal to the 1mm bar on the stage micrometer slide.

Step 3. Calculate a magnification factor for your measurements as follows:

$$\text{Magnification factor} = \frac{1}{\text{Number of divisions counted on eyepiece graticule}}$$

Step 4. Repeat Steps 1–3 for each magnification setting of the microscope that you intend to use for measurements.

How to measure a bee

Step 1. Glue your bee to the tip of a triangular-shaped piece of card mounted on a pin, so you can keep it very still whilst you measure it under the microscope. View the structure of interest through the eyepiece containing the graticule. Count the number of divisions of the eyepiece graticule that are equal to the measurement you wish to make.

Step 2. Calculate the size of the structure as follows:

Size of the structure in millimetres = Number of divisions X Magnification factor.

Here is an example:

Suppose on your microscope at 40x magnification, that 4.05 divisions of the eyepiece graticule are equal to the 1mm bar on the stage micrometer slide. For this microscope at 40x magnification,

$$\text{Magnification factor} = \frac{1}{4.05} = 0.247\text{x}$$

If you were using this microscope to measure the width of a bee's head, and the head width was equal to 6.8 divisions of the eyepiece graticule at 40x magnification, the actual head width would be calculated as follows:

Head width = 6.8 X 0.247 = 1.68 mm.