

## Sexual Communication and Host Plant Associations of Australian Pergid Sawflies (Hymenoptera: Symphyta: Pergidae)

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### Introduction

Pergidae has a Gondwana distribution, with most species known from Australia and South America. A few species occur in North America, but none has been found in Africa. In Australia, most diversity is contributed by the massive radiation that took place within only a few subfamilies, namely the Perginae, Pterygophorinae and Euryinae. Species of the first two subfamilies are almost exclusively associated with host plants of the genus *Eucalyptus* (Table 1), but the trophic relationships of the Euryinae are only poorly known. The few records indicate they are saprophagous or leaf-litter feeding, and some are associated with non-myrtaceous host plants (Table 1).

Eucalypts are adapted to open woodland and only a few grow in stands mixed with rainforest species, where the two vegetation types mix to form wet sclerophyll forests (Adam 1992, p. 88ff.). The eucalypts and their allies apparently evolved in association with the increasingly dry conditions during the Tertiary, and the subsequent contraction of the rainforests that had once covered most of Australia (Kemp 1981). Eucalypts evolved after the geographical isolation of Australia from South America and are confined to Australasia (Ladiges 1997), and this information offers a time frame for interpreting

**Tab. 1:** Species richness and host plant associations of Australian pergid subfamilies. Habitat data of plants taken from Stanley & Ross (1983, 1986).

	Subfamily	No of genera	No. of species	Major host plant genera (minor host plants in brackets)	Habitat of major host plants
Larger subfamilies	Perginae	8	61	<i>Eucalyptus</i> ( <i>Acmena</i> , <i>Melaleuca</i> , <i>Angophora</i> , <i>Syncarpia</i> , <i>Rhodamnia</i> ) (Myrtaceae)	Open woodland
	Pterygophorinae	2	22	<i>Eucalyptus</i> ( <i>Leptospermum</i> , <i>Melaleuca</i> , <i>Callistemon</i> , <i>Syzygium</i> ) (Myrtaceae), <i>Rumex</i> (Polygonaceae)	Open woodland
	Euryinae	8	59	leaf-litter, saprophagous; <i>Eucalyptus</i> , also <i>Marsilea</i> (Marsileaceae), <i>Pennisetum</i> (Poaceae)	variable
	Phylacteophaginae	3	7	<i>Eucalyptus</i>	Open woodland
Smaller subfamilies	Styracotechyinae	1	1	<i>Austrostenisia</i> (Fabaceae)	Creek / river banks, near or in rainforests
	Pteryperginae	1	3	<i>Elaeocarpus</i> (Elaeocarpaceae)	Light rainforest or open forest
	Philomastiginae	1	3	<i>Rubus</i> , <i>Alphitonia</i> (Rhamnaceae)	Mainly in or near rainforests
	Pergulinae	1	1	Unknown	n / a

the evolution of the pergid subfamilies associated with modern Myrtaceous host plants.

Our pergid work has two thrusts, to develop an understanding of their sexual communication and their host plant associations. The work on mating behaviour primarily helps to define species limits, since many species are described as highly variable and having considerable ecological plasticity. Here we detail work on three species. The pergine *Pergagraptia polita* (Leach, 1817), is reputedly variable in morphology and host associations. However, testing for cryptic species within this taxon presented practical challenges, which we detail later. By contrast, the pterygophorine species *Lophyrotoma analis* (Costa, 1864) proved more tractable behaviourally and we detail elements of its sexual communication system (the Specific-Mate Recognition System or SMRS) to illustrate the possibilities of using this approach with the Pergidae (Section I). In Section II we turn to the variable morphology and host plant relationships of *P. polita*. Finally, we describe the host plant relationships of *Styracotechys dicelysma* Benson, 1935 (Section III), the single member of the monotypic subfamily Styracotechyinae as an example of a pergid sawfly that occurs in subtropical rainforests, unlike the Perginae.

## I. Sexual communication and life history traits of *Lophyrotoma analis*

Among sawflies, studies on sexual behaviour, and in particular pheromone studies, have mostly been devoted to the families Diprionidae, Tenthredinidae, and Pamphiliidae (Anderbrandt 1993). To investigate sexual communication in pergids we selected *Lophyrotoma analis*, a species with free-feeding larvae that is comparatively easy to maintain in the laboratory. *Lophyrotoma analis* is widely distributed in Australia and has been recorded in Queensland, New South Wales, ACT, South Australia, Victoria, and Northern Territory (Benson 1938). This wide distribution is probably connected with its feeding on dock (*Rumex* spp.), a widespread weed in Australia. There are 12–16 *Rumex* species in Australia, with 10 species in south-eastern Queensland, where our study was conducted. Four of these 10 local species are native to Australia (Stanley & Ross 1983). According to Radford (in Benson 1938), the larvae of *L. analis* feed also on *Emex australis* Steinh., which is, contrary to Benson's notion, not native to Australia (Gilbey & Weiss 1980). Therefore the original host of *L. analis* is most

probably *Rumex*. The host plants of *L. analis* are exceptional among Pterygophorinae hosts since most species of the subfamily feed on members of the plant family Myrtaceae, in particular *Eucalyptus*, *Callistemon*, *Melaleuca*, and *Syzygium*.

### Material and Methods

Larvae of *Lophyrotoma analis* were collected at Brookfield near Brisbane on swamp dock, *Rumex brownii* Campd., and reared in the laboratory on freshly picked leaves of the same host plant species. The food was changed every day until larvae were ready for pupation. They were then put into glass vials with peat moss and kept at room temperature until the emergence of adults.

#### a. Long-distance attraction

Field bioassays with mated and virgin females were conducted near Brisbane in an area where *L. analis* was locally abundant on swamp dock. The age of virgin and mated females when placed in the field was two days, with the latter mated on the first day after emergence. Different males were used to mate each female and successful mating was confirmed in each case (see under mating behaviour). The females were placed in small containers covered with gauze. The containers were fixed inside “sticky traps” that were made from white cardboard, 25 × 20 cm, with both ends open. The traps were coated on the inner side with Tanglefoot™ and placed in pairs (virgin/mated) with a distance of 50–70 m between pairs, and in a tree about 50 cm above the ground (n = 6 pairs). At no time were traps exposed to direct sunlight. Because males are attracted to the females during the day, the approach flight of males (n = 15) could be observed.

#### b. Cuticular hydrocarbons of virgin and mated females

Cuticular hydrocarbons were extracted by soaking single specimens in n-hexane. Initially, one specimen was extracted in 2 ml of n-hexane for 20 sec followed by a 10 min extraction in 2 ml of n-hexane, keeping each extract separate to determine the effect of extraction time upon the amount of hydrocarbons in the sample. Each lipid extract was concentrated to 0.5 ml and applied to 4 cm of activated silica gel in a

Pasteur pipet minicolumn and the hydrocarbon fraction eluted with 5 ml hexane. The extracted hydrocarbons were evaporated to dryness under nitrogen and redissolved in 100 µl of n-hexane for subsequent GC analysis. The samples were analysed with a Hewlett-Packard 6890 gas-chromatograph equipped with a 30 m HP-5 (5%)-diphenyl-(95%)-dimethylsiloxane copolymer capillary column (0.32 mm internal diameter, 0.25 µm film thickness) with nitrogen as carrier gas. The column was held at 120 °C for 2 min after splitless injection and then the temperature was increased to 240 °C at a rate of 15 °C/min, and after that at a rate of 5 °C/min until the final temperature of 300 °C.

#### c. Behavioural observations on role of female hydrocarbons

Mating experiments were conducted in the laboratory with adults that had been collected as larvae in Brookfield near Brisbane. Only virgin females were used in the tests on the role of cuticular hydrocarbons, to avoid any possible effects of mating experience. Female sawflies were placed alone on a disk of filter paper (90 mm diameter), which was held centrally on a long needle to prevent the insects from escaping as they rarely fly. The male was then placed on the same disk and mating time was measured as the time between the first positive response of the male in relation to the female until the mating partners separated after intromission.

The same setup was used for experiments with dead females, where females were killed by holding them at –60 °C for 10 minutes. After taking the females from the freezer they were left at room temperature for a few minutes before use in a test.

To assess the role of cuticular hydrocarbons mating was interrupted, in a separate set of tests, as soon as the male made contact with the female. The female was then killed by freezing and the mating experiment was repeated with the same male. If the male responded to the dead female then her cuticular hydrocarbons were removed by soaking her briefly in n-hexane. The mating experiment was again repeated to assess whether removal of cuticular hydrocarbons had any effect on male behaviour.

#### d. Antennal sensillar equipment of males and females

For scanning electron microscopy, the heads of adults were removed, dehydrated through an ethanol series (60, 70, 80, and 90%), and left overnight in absolute ethanol. The specimens were covered in hexamethyldisilazane (HMDS), left to dry completely (Braet 1997) and attached to a specimen holder and sputter-coated with gold for 90 seconds. The material was examined in a Jeol 6400 scanning electron microscope (SEM).

### Results

#### a. Long distance attraction

Only males were found in the traps at the end of the experiment, and traps with virgin females attracted significantly more males than traps with mated females (56 vs 2 males, respectively,  $p < 0.02$ ,  $n = 12$ , Mann-Whitney U-test). No females were collected in the traps. When the experiment was terminated after five days, all females were still alive. One virgin female did not attract any males, whereas the maximum number of males attracted by a single virgin female was 23 (Table 2).

Our behavioural observations in the laboratory and in the field show that the searching behaviour of males was clearly directional in relation to the source of the sex attractant. Males hovered near the pheromone traps before approaching them to make contact with them. On the substrate near the female, searching seemed to be more random, with males walking in the vicinity of the female but not straight towards her from the outset. This behaviour indicated that the physical localisation of the

female took place only at very close range, and that probably close-up recognition took place only after physical contact. Similar behaviour has been described for the yellow-headed spruce sawfly, *Pikonema alaskense* (Rohwer) (Bartelt et al. 1982).

Extraction and analysis of pheromones by headspace sampling and subsequent gas-chromatographic analysis proved fruitless, probably because pheromonal compounds are produced in small amounts (J.-L. Boevé, pers. comm.). The only sawfly pheromones that have so far been identified positively were present only in nano quantities (Jewet et al. 1976, Bartelt et al. 1983). Thus, possible peaks from the *L. analis* headspace samples were probably too small to be separated from background. Electroantennographic analysis combined with gas chromatography procedures (GC-EAG) would be needed. The strongly forked antennae of the *L. analis* males (see Fig. 6d), which have a relatively large surface area, provide another indication that the pheromone concentrations emitted from the female are extremely low and/or that pheromones can be detected by males at a relatively long distance.

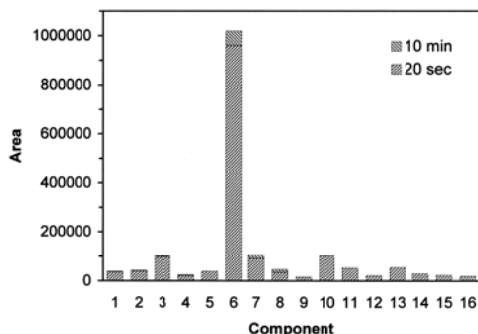
#### b. Cuticular hydrocarbons

Gas chromatographic analysis of *L. analis* cuticular extracts revealed 16 major components that were investigated further for the efficiency of the extraction method (Fig. 1). In particular, we found that soaking a specimen of *L. analis* for 20 seconds in n-hexane removed between 90 and 100% of the cuticular hydrocarbons in most cases (Fig. 2). In only two of the 16 components was less than 80% extracted. The efficiency of an extraction lasting 20 sec was confirmed by the subsequent extended (10 min) soaking of each specimen, by means of which very little additional hydrocarbon material was recovered (Fig. 1). Since a 20 sec extraction removed virtually all hydrocarbons from the cuticle this extraction time was used for all subsequent experiments. Such a short extraction time also reduces the risk of extracting unwanted components from internal tissues.

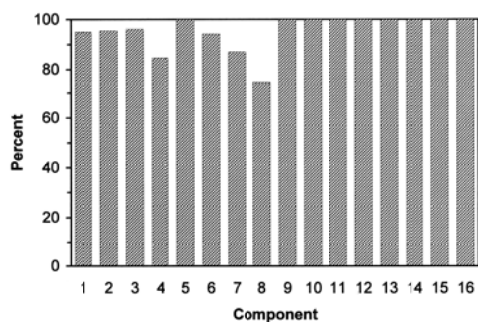
The concentration of cuticular hydrocarbons increased with age in virgin females. Within five days of emergence they had increased more than 2.5 times, whereas in mated females there was only a small change with age (Fig. 3). At

**Tab. 2:** Number of males attracted to pheromone traps, each with one virgin or mated female of *Lophyrotoma analis*.

Replicate	Virgin female	Mated female
1	23	1
2	3	0
3	4	0
4	5	1
5	0	0
6	21	0
Total:	56	2



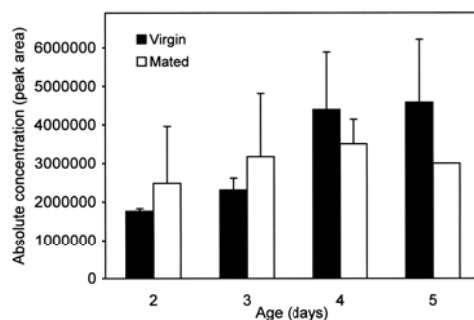
**Fig. 1:** Amount of cuticular hydrocarbons of 16 components, expressed as area under the peak of each component on a GC-trace, after washing specimens of *Lophyrotoma analis* for 20 seconds and then again for 10 minutes in n-hexane.



**Fig. 2:** Relative yield of cuticular hydrocarbons after soaking a specimen of *Lophyrotoma analis* for 20 seconds in n-hexane.

**Tab. 3:** Retention times and equivalent chain length (E.C.L.) of major cuticular hydrocarbons (see Fig. 4) of virgin and mated females of *Lophyrotoma analis*.

Component	Retention time (sec)	E.C.L.
21	15.185	27.17
22	15.531	27.50
23	15.881	27.84
25	16.253	28.19
27	16.968	28.84
29	17.378	29.20
30	17.785	29.55
32	18.125	29.83
36	19.213	30.71
39	20.082	31.38



**Fig. 3:** Change of concentrations of cuticular hydrocarbons of virgin and mated females with ages between two and five days after emergence (average amounts of total cuticular hydrocarbons, expressed as area under the peaks on a GC-trace). Hydrocarbon concentrations of virgin females vary significantly with age ( $p < 0.05$ ,  $n = 11$ , Kruskal-Wallis test), whereas concentrations of mated females do not show significant differences ( $p < 0.80$ ,  $n = 10$ ). Number of replicates for virgin females: 2 days:  $n = 2$ , 3 days:  $n = 3$ , 4 days:  $n = 3$ , 5 days:  $n = 3$ ; mated females: 2 days:  $n = 3$ , 3 days:  $n = 3$ , 4 days:  $n = 3$ , 5 days:  $n = 1$ , error bars indicate 1 SD).

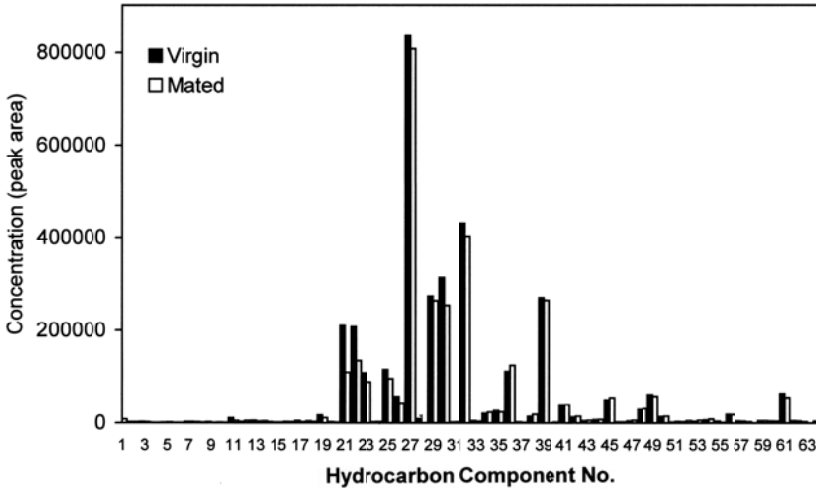
day five, virgin females had about 1.5 times more cuticular hydrocarbons than mated ones (Fig. 3).

GC-analyses of n-hexane extracts of 11 virgin and 10 mated females of different age classes revealed 64 hydrocarbons of which the concentration profiles are shown in Fig. 4. No qualitative and only minor quantitative differences were detected in a few components between virgin and mated females (Fig. 4). Virgin females had higher concentrations of some of the relatively

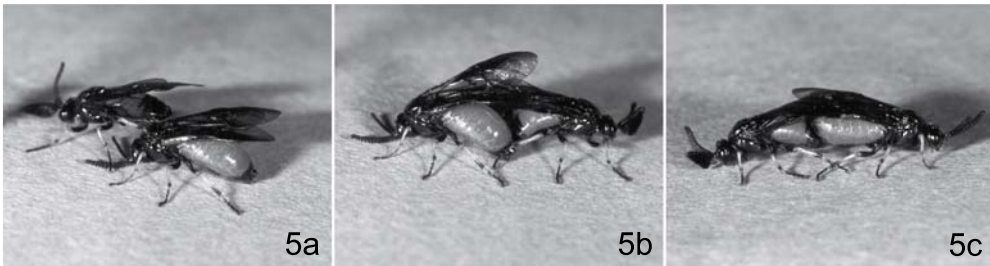
more volatile components, components 21 and 22 in particular. Some of those of longer chain length showed a slightly higher concentration in virgin females, but from component 34 upwards only minor differences were evident (Fig. 4). By using hydrocarbon standards, the equivalent chain lengths (E.C.L., equivalent to number of carbon atoms) of components 21 and 22 could be identified as 27.17 and 27.50, respectively (Table 3). The E.C.L.'s of the 10 major components are given in Table 3.

**Tab. 4:** Mating experiment with *Lophyrotoma analis* females that had had their cuticular lipid components gradually removed by a solvent (experiments 1–4) and then reapplied (experiment 5). Males 1 and 2 were 6 days old, the other males 2 days old. Coding of male behaviour: - no response; (+) slight response, i.e. only step 1 or step 1 and 2 (see text and Fig. 3), then losing “interest”; + normal sequence from step 1 to step 3.

Experiment No.	Experiment	Male No.					p (Wilcoxon matched-pairs test)
		1	2	3	4	5	
1	♀ tested with every ♂	+	+	+	+	+	-
2	♀ washed 3 x in 1 ml hexane (1 min)	-	-	+	+	+	-
3	♀ washed 2 x in 1 ml hexane (1 min)	-	-	(+)	(+)	(+)	n.s. (Exp. N°. 3 vs 1)
4	♀ washed 10 x in 1 ml hexane and 2 x in 1 ml acetone (10 sec)	-	-	-	(+)	-	p<0.05 (Exp. N°. 4 vs 1)
5	Hexane extract concentrated and reapplied to ♀	(+)	(+)	+	+	+	p<0.05 (Exp. N°. 5 vs 4)



**Fig. 4:** Concentrations of 64 cuticular hydrocarbons found by gas-chromatographic analyses of virgin and mated females of *Lophyrotoma analis*. The concentration is given as absolute area of a peak of a particular component on a GC-trace.



**Fig. 5:** Mating of *Lophyrotoma analis*, a: male aligns to female, b: male attaches apex of abdomen to the female abdomen, c: mating position (see also Colour Plate 16).

### c. Behavioural observations on role of female hydrocarbons

A male that was placed on a disc together with a female did not move directly towards the female but seemed to encounter the female by chance. Starting with the male making physical contact with the female, usually by means of his antennae, the mating sequence of *L. analis* can be divided into three sequential steps. Immediately after initial contact (step 1, Fig. 5, a), the female stopped moving and adopted a specific mating posture. Her body was arched upwards slightly, with the apex of the abdomen and the head very close to the substrate and with the wings slightly raised. This posture was maintained during mating. Females also exhibited this behaviour when they were picked up with forceps. The male then started to align itself in a position 180° opposite to the position of the female. This is typical for strophandrious sawflies, which have their male genitalia twisted 180° along the longitudinal axis (Benson 1950). The male then tried to position his abdominal apex in apposition to that of the female (step 2, Fig. 5, b). This entire procedure lasted only a few seconds. Once a successful connection was established, both insects remained in the same position, genitalia locked and without movement, until mating was finished (step 3, Fig. 5, c). With successful attachment of the pair, the female released her initial arched posture (cf. Fig. 5, c). After disconnection the male often left the mating place before the female moved away. Successful mating lasted on average 6.7 min (5.6–7.9 min, SD = 0.6 min, n = 22).

Males tried to mate with the females to which they had originally responded, but which had recently been killed by freezing (experiment 1, Table 4). To investigate the role of cuticular hydrocarbons, the mating sequence was interrupted at step 1 or 2, and the hydrocarbons were removed from the female cuticle by several means (see Methods). Five males were tested in this way and the behaviour of the male was observed (Table 4). Three of the five responded positively to the female after she was washed three times in n-hexane (experiment 2, Table 4), indicating that hexane did not remove all chemical cues from the female. The female was then washed twice again in n-hexane (experi-

ment 3, Table 4) before being re-tested. After that she was washed 10 times in n-hexane and twice in acetone (experiment 4, Table 4). Acetone is a much better solvent for polar components and the acetone washing possibly removed components less volatile than hydrocarbons, which were only partly removed by the hexane treatment. Each subsequent washing protocol reduced the response until finally only one of the males showed a weak response (experiment 4, Table 4). After concentrating and re-applying the n-hexane extracts of the previous experiments to the female from which it had been extracted, all five males showed at least a slight response to the female (experiment 5, Table 4).

### d. Antennal sensillar equipment of males and females

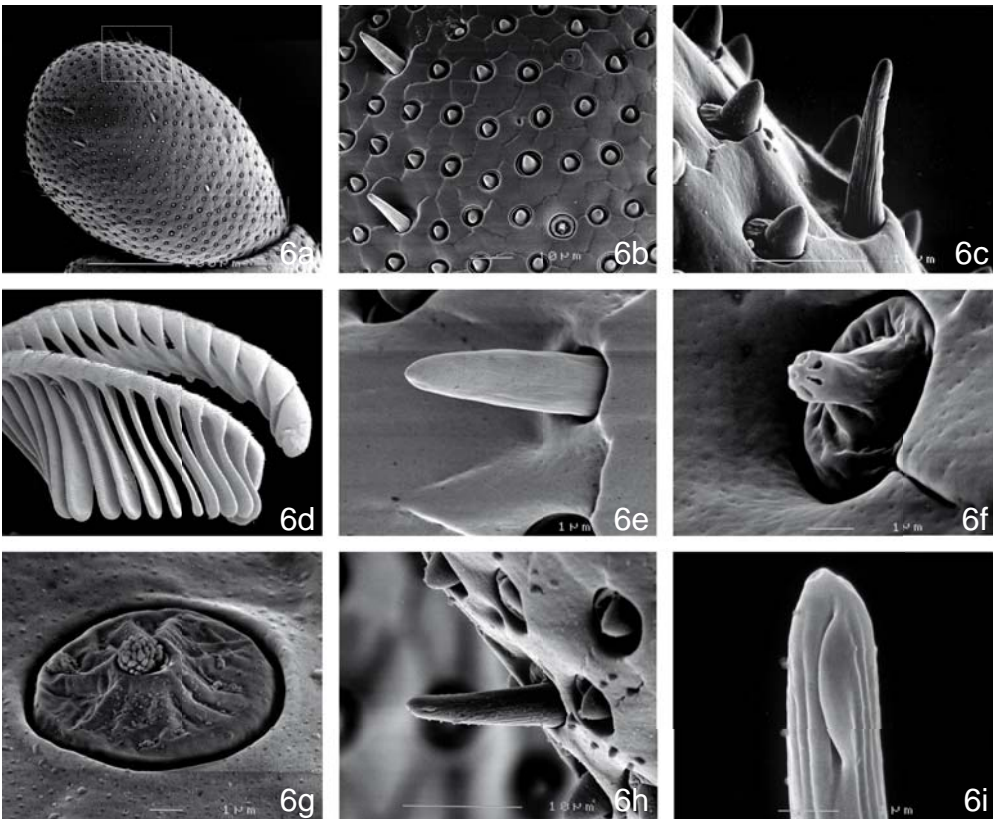
Five different types of sensillar structures could be differentiated on the antennae of males and females and there were no apparent qualitative differences between the sexes. By contrast, Diprionidae have different types of sensilla between the sexes, at least in *Neodiprion sertifer* (Geoffroy, 1785) (Hallberg 1979). Most types of sensilla could be identified on the basis of their close similarities to those of other sawflies, in particular those of species in the family Diprionidae (Hallberg 1979, Anderbrant et al. 1995). They are named according to the terminology of Altner (1977), who classified insect sensilla according to their external morphology. The main differences between *L. analis* and *N. sertifer* is the absence of long single-walled and medium-long single-walled sensilla in *L. analis*. Both types are sex-specific, with the long single-walled sensilla characteristic for males, whereas the medium-long single-walled sensilla are present only in females (Hallberg 1979). The long single-walled sensilla in males apparently serve as pheromone receptors (Hallberg 1979).

The most common sensilla on the antennae of *L. analis* are present both in males and females and are different from any of the sensilla so far found in Diprionidae. These sensilla are rather short, dome-shaped, more or less retracted and almost uniformly distributed on all antennal surfaces (Fig. 6, b, c, h). In some areas of the antenna the apex of these “dome-shaped sensilla” hardly extends to the external cuticle of

the antennal surface (Fig. 6, b, h). All other sensilla are present only in low numbers. These include an unspecified type of sensillum (Fig. 6, e), a double-walled sensillum (Fig. 6, f), a non-pore sensillum (Fig. 6, g), and a terminal-pore sensillum (Fig. 6, h, i). The last of these has, in addition to the terminal pore, subapical longitudinal slit-like pores (Fig. 6, i). Despite the qualitative similarity between the antennal sensilla across the sexes, the males have many more than females, especially of the most common type (Fig. 6, b, c, h), which is mainly related to the larger surface area of the flabellate male antenna (Fig. 6, d).

#### e. Oviposition behaviour and larval development

*Lophyrotoma analis* is polyvoltine in the Brisbane area and in suitable habitats larvae can be found almost all year except for a short period in winter. After completing their development the larvae enter the soil and prepare a cocoon where they develop to the adult stage within 13–22 days (mean = 15.5, SD = 1.75, n = 161). Males lived for 6 to 10 days (mean = 8.0, SD = 1.9, n = 15) and females 3 to 9 days (mean = 5.1, SD = 2.2, n = 10) with access to water and honey. All of these data derive from the laboratory at room temperature (Brisbane,



**Fig. 6:** Types of sensilla on the antennae of male and female *Lophyrotoma analis*. **a:** apical segment of female antenna; **b:** magnification of the rectangular area shown in **a** with two long unspecified sensilla, one double-walled sensillum (bottom right), and numerous retracted sensilla of the most common type (“dome-shaped sensilla” in the text); **c:** female antennae with one terminal pore sensillum and several dome-shaped sensilla; **d:** male antenna, **e:** unspecified type of sensillum; **f:** double-walled sensillum; **g:** non-pore sensillum; **h:** long terminal-pore sensillum and several retracted dome-shaped sensilla; **i:** magnification of apex of terminal pore sensillum.



**Fig. 7:** Female of *Lophyrotoma analis* ovipositing on swamp dock (*Rumex brownii*) (see also Colour Plate 16).



**Fig. 8:** Full grown larva of *Lophyrotoma analis* feeding on dock (*Rumex brownii*) (see also Colour Plate 16).

in summer). About one fifth of field-collected larvae (22.2%,  $n = 207$ ) was parasitised by Ichneumonidae (2.9%) and Tachinidae (19.3%).

Females place their eggs into the leaf margin of their host plant. The eggs are laid singly, not in clusters as in species of the subfamily Perginae, although a leaf may receive several eggs. When a female is placed on a dock leaf it almost invariably starts immediately to examine the surface with its ovipositor tip, by bending the abdomen. It then uses the antennae to examine the leaf surface and moves to the leaf margin where it usually starts ovipositing at once (Fig. 7).

The larvae are morphologically similar to the larvae of the family Argidae. As in argids the body is laterally broadened ventrally and lacks any apical or dorsal appendages (Fig. 8). The head is orange-brown and the body has a whitish appearance and the internal organs and gut contents shine through the translucent cuticle, giving the larva a slightly greenish colour. Only the first thoracic segment dorsally and the abdominal apex differ in colour, being more or less yellow. The entire body is covered with black spots (Fig. 8).

## Discussion

Chemicals that act as long distance attractants have been found in sawfly species in the families Diprionidae, Tenthredinidae and Pamphiliidae (Longhurst & Baker 1980, Borden et al. 1978). We report the first behavioural evidence for the existence of a distance sex pheromone in the family Pergidae. Virgin females of *L. analis* produce a pheromone that attracts males,

whereas mated females lose this aspect of their sexual attractiveness (Table 4). This agrees with observations on other sawfly species: males of *Cephalcia abietis* (Linné, 1758) (Pamphiliidae) respond to virgin females from a distance but to mated females only when they are confined in small cages (Gruppe 1996). In the tenthredinid sawfly *Nematus ribesii* (Scopoli, 1763), mated females attracted very few males compared to traps baited with virgin females (Longhurst 1980).

That males do mate readily with mated females, at least when put together in the laboratory, indicates that at close distance other pheromones are responsible for the recognition of a potential mate. Our mating experiments demonstrate that chemical components of the female cuticle play a major role in the close-up recognition of a potential mate by males of *L. analis*. Indeed, males' response to females decreased after the latter had been soaked in an organic solvent, but increased again when the extracts were re-applied on the female (Table 4).

Visual and acoustic signals apparently have only little importance, if any at all, in the mating process. Males tried to mate with females that had been killed shortly before, by freezing, and their response was no different from that to living females. This suggests that males do not actively recognise or respond to the mating posture adopted by living females and that acoustic signals may not be exchanged. The mating posture of the female may, therefore, play some other role in mating, perhaps to do with sperm transfer to the female and/or

sperm movement within her internal genitalia. The behaviour of *P. analis* contrasts with that of the pamphiliid sawfly *Cephalcia lariciphila* Wachtl, 1898 whose males mate only with living females (Baker et al. 1983).

The mating behaviour observed in *L. analis*, with the male being much more active than the female, is typical for most sawflies (Benson 1950). Based on the behaviour of males before and during successful mating, the process can be summarised into several consecutive steps: 1) long-distance pheromonal attraction of males to females, 2) precise localisation and subsequent recognition of the female by the male through antennal contact with the cuticular hydrocarbons, 3) female arching her body in response to male contact, 4) male turning 180°, assessing and aligning his body to the lengthwise orientation of the female, 5) male aligning himself lengthwise, facing away from the female to make contact between their abdominal tips, and 6) engaging their strophandrious genitalia.

## II. Host plant specificity and intraspecific variation in *Pergagraptia polita*

Currently the subfamily Perginae includes 61 described species (see Schmidt, 2006), but host plants have been recorded for only one third of them (Table 5). Despite their relatively large size, perginines are only rarely collected as adults and many of the species are represented by only a few specimens in collections, and in some cases only the type specimen is known. Even some of the most common species, like *Perga affinis* Kirby, 1882 and *P. dorsalis* Leach, 1817 are mostly encountered in the field as larvae and only occasionally adults are found, indicating that the rarity may be largely a reflection of the cryptic behaviour of the adults. Males and females of all perginines have a well developed flight apparatus and some species are known to be very strong flyers. Furthermore, behavioural observations show that they tend to spend most of the time in the canopy area of their host plant, so they are generally out of sight of collectors.

The known host plant range of common perginine species is considerably larger than that of the rare perginines, so it is possible that the strict monophagy recorded for many species

(Table 5) is a consequence of their rarity rather than an accurate reflection of their ecology. Furthermore, most literature records include only perginine species of economic importance, such as *P. affinis*, *P. dorsalis*, and *P. polita* (Table 5). Perginae are closely associated with the plant family Myrtaceae and major host plants include eucalypts (*Eucalyptus* spp. and *Corymbia* spp.) whereas only a few species feed on other plants of the family Myrtaceae, e.g. *Melaleuca*, *Angophora*, and *Acmena* (Table 5).

*Pergagraptia polita* (Leach, 1817) is a common species in eastern Australia and occurs from Queensland south to New South Wales and Victoria (Morice 1919, Forsius 1929, Benson 1939, Muche 1986, Purcell & Goolsby 2005). Larvae of *P. polita* have been recorded from more than a dozen *Eucalyptus* species, *Angophora floribunda*, and *Melaleuca quinquenervia* (Table 5). The larvae feed on young foliage and after completing their development they enter the ground for pupation, as in all other perginines investigated to date.

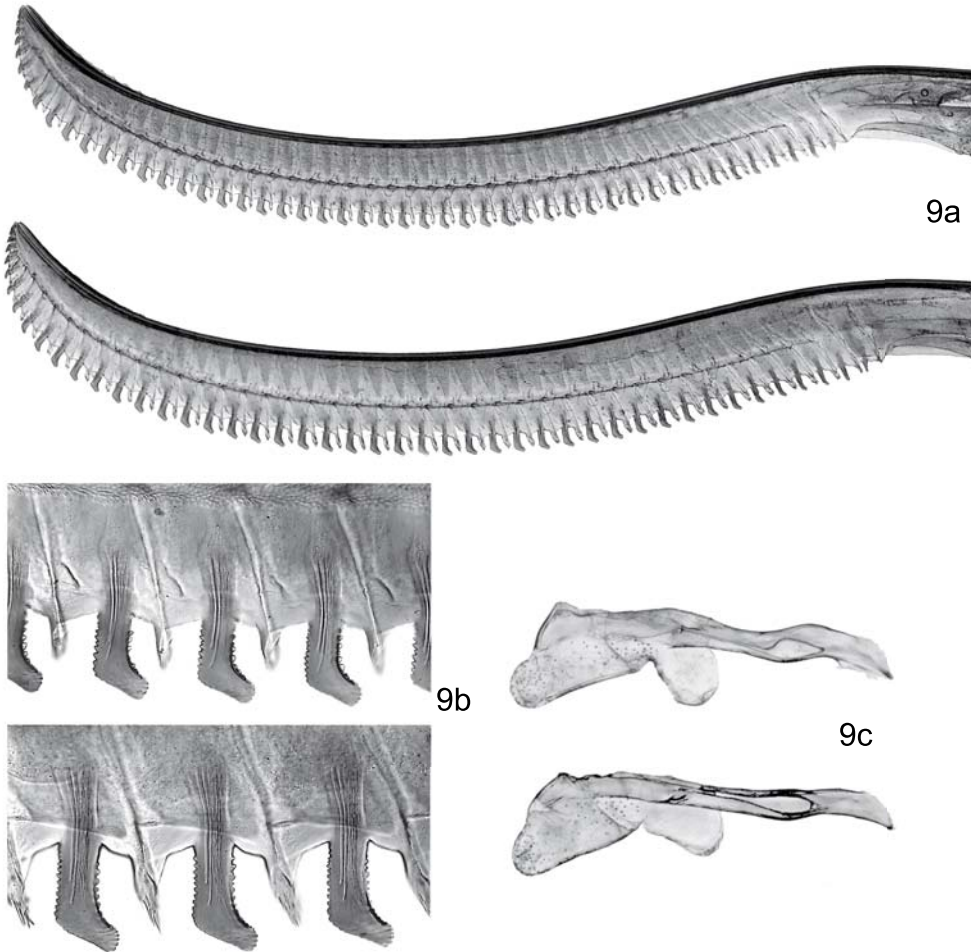
The eucalypt host plants of *P. polita* and other perginines contain high amounts of potentially toxic essential oils. These oils are mechanically extracted from nutritive parts of the leaf by means of a specialised morphological structure of the mandible, the mandibular brush, and are stored unmodified in a pharyngeal diverticulum (Carne 1962, Morrow et al. 1976, Schmidt et al. 2000). These stored oils are emitted under two circumstances. Field experiments and laboratory observations showed that oil from the diverticulum is voided prior to and/or during feeding at night, which indicates that the primary function of this mechanism is to eliminate host associated oils (Schmidt et al. 2000). During the day larvae rest in close aggregations and during this time they retain a full diverticulum, and the oils may be emitted for defensive purposes when larvae are disturbed (Bennet & Scott 1859, Westwood 1880, Froggatt 1901).

That *P. polita* feeds on paperbark (*M. quinquenervia*) has been known for some time, because of its interest to biological control efforts in Florida (Purcell & Goolsby 2005). Its identity has been treated as uncertain because no perginine species is so far known to have a host range that encompassed both eucalypts and paperbarks (see above), which fall into dif-

**Tab. 5:** Host plant associations of species of the subfamily Perginae. The table includes literature records and personal observations of the authors (Bennet & Scott 1859<sup>1</sup>; Bennet 1860<sup>2</sup>; Brittlebank 1888<sup>3</sup>; Froggatt 1890<sup>4</sup>, 1899<sup>5</sup>, 1901<sup>6</sup>, 1916<sup>7</sup>, 1918<sup>8</sup>; Benson 1939<sup>9</sup>; Moore 1972<sup>10</sup>; Leask 1946<sup>12</sup>; McKeown 1951<sup>13</sup>; Carne 1962<sup>14</sup>, 1965<sup>15</sup>, 1969<sup>16</sup>; Morrow et al. 1976<sup>17</sup>; Rose 1987<sup>18</sup>; Jones & Elliot 1990<sup>11</sup>; Weinstein 1990<sup>19</sup>; Elliot & Bashford 1995<sup>20</sup>; Weinstein & Austin 1995<sup>26</sup>; Neumann & Collett 1997<sup>25</sup>; Weinstein & Maelzer 1997<sup>21</sup>; Elliot et al. 1998<sup>22</sup>; Purcell & Goolsby 2005<sup>23</sup>; personal observations of the authors<sup>24</sup>).

Species	Host plant
<i>Acanthoperga cameronii</i> (Westwood, 1880)	<i>Corymbia gummifera</i> <sup>5</sup>
<i>Acanthoperga marlatti</i> Benson, 1939	<i>Acmena smithi</i> <sup>23</sup>
<i>Antipergera enslini</i> Benson, 1939	<i>Melaleuca</i> sp. (probably) <sup>9</sup>
<i>Cerealesc scutellata</i> Kirby, 1882	<i>Eucalyptus pauciflora</i> subsp. <i>niphophila</i> <sup>17</sup>
<i>Perga affinis</i> Kirby, 1882*	<i>Corymbia ficifolia</i> <sup>20</sup> , <i>Eucalyptus amygdalina</i> <sup>20</sup> , <i>E. bicostata</i> <sup>15,16</sup> , <i>E. blakeyi</i> <sup>4,15,16,22</sup> , <i>E. bridgesiana</i> <sup>15,16</sup> , <i>E. camaldulensis</i> <sup>14,15,16,22</sup> , <i>E. elaeophora</i> <sup>15</sup> , <i>E. globulus</i> <sup>20,22</sup> , <i>E. goniocalyx</i> <sup>25</sup> , <i>E. grandis</i> <sup>20</sup> , <i>E. haemastoma</i> <sup>25</sup> , <i>E. lansdowneana</i> <sup>20</sup> , <i>E. leucoxydon</i> <sup>20</sup> , <i>E. mannifera</i> <sup>14,15,16,22</sup> , <i>E. melliodora</i> <sup>14,15,16,22</sup> , <i>E. nicholii</i> <sup>20</sup> , <i>E. nitens</i> <sup>20,22</sup> , <i>E. obliqua</i> <sup>20,22</sup> , <i>E. occidentalis</i> <sup>25</sup> , <i>E. ovata</i> <sup>20,22</sup> , <i>E. pauciflora</i> <sup>25</sup> , <i>E. polyanthemosa</i> <sup>15</sup> , <i>E. rubida</i> <sup>15,16</sup> , <i>E. sideroxylon</i> <sup>15</sup> , <i>E. viminalis</i> <sup>16, 20, 22</sup> .
<i>Perga dahlbomii</i> Westwood, 1880	<i>Angophora floribunda</i> <sup>23</sup> , <i>Eucalyptus lehmanni</i> <sup>23</sup>
<i>Perga dorsalis</i> Leach, 1817	<i>Callistemon</i> sp. <sup>2</sup> , <i>Corymbia citriodora</i> <sup>1,2,12</sup> , <i>C. gummifera</i> <sup>6,12,24</sup> , <i>Eucalyptus camaldulensis</i> <sup>24</sup> , <i>E. globulus</i> <sup>6</sup> , <i>E. grandis</i> <sup>10</sup> , <i>E. haemastoma</i> <sup>24</sup> , <i>E. leucoxydon</i> <sup>26</sup> , <i>E. nova-anglica</i> <sup>7,8,12</sup> , <i>E. obliqua</i> <sup>24</sup> , <i>E. obtusiflora</i> <sup>4,12</sup> , <i>E. ovata</i> <sup>11</sup> , <i>E. sieberi</i> (rarely) <sup>23</sup> , <i>E. torquata</i> <sup>19</sup> , <i>E. umbra</i> <sup>24</sup>
<i>Perga kirbii</i> Leach, 1817	<i>Corymbia maculata</i> <sup>22,24</sup> , <i>Eucalyptus camaldulensis</i> <sup>22</sup> , <i>E. grandis</i> <sup>11</sup>
<i>Perga klugii</i> Westwood, 1880	<i>E. globulus</i> <sup>24</sup>
<i>Perga konowi</i> Benson, 1939	<i>E. transcontinentalis</i> <sup>9</sup>
<i>Perga schiodtei</i> Westwood, 1880	<i>Corymbia calophylla</i> <sup>24</sup> , <i>Eucalyptus globulus</i> <sup>24</sup>
<i>Perga vollenhovii</i> Westwood, 1880	<i>E. crebra</i> <sup>24</sup>
<i>Pergagraptia bella</i> (Newman, 1841)	<i>Corymbia gummifera</i> <sup>4,12</sup> , <i>Eucalyptus amygdalina</i> <sup>20,22</sup> , <i>E. pauciflora</i> <sup>20,24</sup> , <i>E. piperita</i> <sup>24</sup> , <i>E. viminalis</i> <sup>20,22</sup>
<i>Pergagraptia bicolor</i> (Leach, 1817)	<i>Corymbia gummifera</i> <sup>4,24</sup> , <i>Eucalyptus haemastoma</i> <sup>24</sup> , <i>E. pauciflora</i> <sup>24</sup> , <i>E. saligna</i> <sup>24</sup>
<i>Pergagraptia castanea</i> (Kirby, 1882)	<i>E. piperita</i> <sup>24</sup>
<i>Pergagraptia glabra</i> (Kirby, 1882)	<i>E. crebra</i> <sup>24</sup> , <i>E. tereticornis</i> <sup>24</sup>
<i>Pergagraptia gravenhorstii</i> (Westwood, 1880)	<i>E. nicholii</i> <sup>24</sup> , <i>E. sideroxylon</i> <sup>24</sup>
<i>Pergagraptia latreillii</i> (Leach, 1817)	<i>E. leucoxydon</i> <sup>24</sup> , <i>E. perriniana</i> <sup>16</sup> , <i>E. punctata</i> <sup>24</sup>
<i>Pergagraptia polita</i> (Leach, 1817)	<i>A. floribunda</i> <sup>24</sup> , <i>Corymbia citriodora</i> <sup>24</sup> , <i>C. gummifera</i> <sup>4</sup> , <i>Eucalyptus bicostata</i> <sup>24</sup> , <i>E. botryooides</i> <sup>24</sup> , <i>E. crebra</i> <sup>24</sup> , <i>E. deanei</i> <sup>24</sup> , <i>E. elata</i> <sup>24</sup> , <i>E. bicostata</i> <sup>24</sup> , <i>E. globulus</i> <sup>18</sup> , <i>E. grandis</i> <sup>10,24</sup> , <i>E. melliodora</i> <sup>24</sup> , <i>E. moluccana</i> <sup>24</sup> , <i>E. nicholii</i> <sup>24</sup> , <i>E. parramattensis</i> <sup>18</sup> , <i>E. pilularis</i> <sup>24</sup> , <i>E. propinqua</i> <sup>24</sup> , <i>E. saligna</i> <sup>24</sup> , <i>E. scoparia</i> <sup>24</sup> , <i>E. sideroxylon</i> <sup>24</sup> , <i>Melaleuca quinquenervia</i> <sup>23,24</sup>
<i>Pergagraptia spinolae</i> (Westwood, 1880)	<i>Corymbia gummifera</i> <sup>4,12</sup> , <i>C. maculata</i> <sup>24</sup> , <i>Eucalyptus leucoxydon</i> <sup>24</sup> , <i>E. punctata</i> <sup>24</sup> , <i>E. sideroxylon</i> <sup>24</sup> ,
<i>Pergagraptia turneri</i> Benson, 1939	<i>Eucalyptus grandis</i> <sup>10</sup>
<i>Pseudoperga ferruginea</i> (Leach, 1817)	<i>Corymbia gummifera</i> <sup>24</sup> , <i>Eucalyptus crebra</i> <sup>24</sup> , <i>E. propinqua</i> <sup>24</sup>
<i>Pseudoperga guerinii</i> (Westwood, 1880)	<i>Eucalyptus dives</i> <sup>24</sup> , <i>E. pauciflora</i> subsp. <i>niphophila</i> <sup>17,24</sup> , <i>E. piperita</i> <sup>24</sup>
<i>Pseudoperga lewisii</i> (Westwood, 1837)	<i>Corymbia gummifera</i> <sup>5,6,11</sup> , <i>Eucalyptus dives</i> <sup>24</sup> , <i>E. nitens</i> <sup>20</sup> , <i>E. obliqua</i> <sup>4</sup> , <i>E. pauciflora</i> <sup>13</sup> , <i>E. tereticornis</i> <sup>24</sup> , <i>E. viminalis</i> <sup>20, 22</sup>
<i>Xyloperga forsiusi</i> Benson, 1939	<i>Eucalyptus pauciflora</i> subsp. <i>niphophila</i> <sup>17</sup> , <i>E. stellulata</i> <sup>17</sup>

\* Preferred hosts are, according to the large scale studies of Carne (1965, 1969) on *Perga affinis affinis*, *Eucalyptus blakeyi*, *E. camaldulensis*, and *E. melliodora*, whereas *E. bridgesiana* and *E. rubida* suffered severe attack only where they grew near a preferred species. Several other eucalypts in the vicinity of favoured hosts were attacked, including *E. maculosa*, *E. elaeophora*, *E. sideroxylon*, *E. polyanthemosa*, and *E. bicostata*. The following species were never infested, even if in close proximity to preferred hosts: *E. macrorrhyncha*, *E. rossi*, *E. woollisiana*, and *E. albens*.



**Fig. 9:** Genitalia of *Pergagraptia polita*. In each case the upper diagram represents the eucalyptus-feeding insects and the bottom one the paperbark-feeding ones. a: valvula 1, lateral view, b: middle part of valvula 1, lateral view, c: penis valve, lateral view.

ferent major groups within Myrtaceae. This species has consequently been referred to as *Pergagraptia* sp. in published accounts (e.g. Schmidt et al. 2000, Purcell & Goolsby 2005). Our morphological comparisons of adults and larvae have revealed no consistent morphological differences between representatives feeding on eucalypt and paperbark. Even the structure of the genitalia (Fig. 9) is indistinguishable across insects from both plants. The larvae are structurally uniform but the colour is somewhat variable with the base colouring of the body ranging from yellow to dark brown. There is no indication that colouration is affected by

the host plant, since different colour forms have been collected on the same host plant, *M. quinquenervia* (M. Purcell, pers. comm.).

For the reasons outlined above we include the *Melaleuca*-feeding insects under the name *P. polita*. We stress, however, that such unusual host relationships for a single pergid species still suggest several alternative explanations. The question that needs to be resolved first is whether the two host-associated populations represent cryptic species. These are species that are reproductively independent of one another, often ecologically different from one another, and yet may be morphologically indistinguish-

able (Paterson 1991, Walter 2003). To resolve this issue it would be necessary to conduct a detailed investigation of the Specific Mate Recognition System (SMRS) of these sawflies. Our studies demonstrate that in another pergid species (*L. analis*, see above) volatile olfactory components play a major role in the early stages of the recognition of a potential mate. In *P. polita* mating and oviposition experiments were not conducted because the species cannot yet be maintained in the laboratory. In the most comprehensive behavioural study on pergids to date, Carne (1962) found that virgin females did not oviposit in captivity, even in cages up to room size. The other pergid species that have been investigated are apparently similar in this regard. The preliminary long-distance attraction experiments we conducted with *P. polita*, in which live virgin females were placed in pheromone traps in the field (as for *L. analis*, see above), did not attract any males and need to be repeated with more females (and also with males as “bait”) and over a longer period.

Another problem that needs to be clarified before detailed studies on the SMRS can be conducted is the claim that amphitokous parthenogenesis (i.e. a form of reproduction in which progeny are male and female) is the usual mode of reproduction in *Perga affinis* and perhaps even across all Perginae (Carne 1962, Macdonald & Ohmart 1993). Carne (1962) dissected eggs from *P. affinis* and observed normal embryonic development. Since he did not rear them to the adult stage, he could not have sexed them. However, they are likely all to have been males, which is typical for the arrhenotokous parthenogenesis (see summary of Walter (1983)) that characterises the Hymenoptera. No truly amphitokous species is known to produce males at the rate that Carne claimed for *P. affinis*, which suggests that he overlooked the possibility of arrhenotoky in favour of amphitoky. Only a few observations have been made on the sexual behaviour of pergines (e.g. Wilson 1932, J. G. pers. obs.), so there is still no clear evidence that sexuality does actually occur widely in the Perginae. One of us (J. G.) observed freshly emerged males of *Perga dorsalis* attempting to mate with females in captivity, with the females showing little “interest”. It is common among all sawflies, though, that

females often seem “disinclined” to copulate with the males (Benson 1950).

In contrast to adults, larvae of several species are relatively easy to collect in the field and to rear to the adult stage in the laboratory. Once the issues mentioned above have been tackled, then subsequent experiments with *P. polita* need to test whether olfactory components play a role in long distance attraction and close-up recognition. For this purpose field experiments can be conducted using pheromone traps with virgin and mated females as described under *L. analis* (see above). If long distance attraction is mediated by volatile chemical components, then pheromone analyses and electroantennograms could identify the components that constitute an essential part of the SMRS.

### III. Host plant relationships of *Styracotechys dicelysma*

The subfamily Styracotechyinae was erected by Benson (1935) for a single species, *Styracotechys dicelysma* Benson, 1935. The species was described on the basis of a single female collected in 1926 by H. Hacker. For over half a century the species was known only by this female type specimen, deposited in the Queensland Museum, Brisbane. Only recently has the host plant of *S. dicelysma* been discovered (by J. G.). Larvae are found regularly, sometimes in large numbers, feeding on *Austrosteenisia* (= *Kunstleria*) *blackii* (F.Muell.) Geesink (Fabaceae). *Austrosteenisia blackii* is a vine that grows in tropical rainforests along the eastern coast of Australia and in Papua New Guinea, according to the Australian Virtual Herbarium ([www.anbg.gov.au/chah/avh/index.html](http://www.anbg.gov.au/chah/avh/index.html)). The observations reported below were conducted with material collected in the Brisbane area of Queensland, about 130 km NNE of the type locality of the species (Tooloom, New South Wales). The species was also recorded from Lamington National Park in Queensland (S. S., pers. obs.). At the same locations one of us (J. G.) found similar looking larvae on the vine *A. glabristyla* Jessup.

We agree that the subfamily status accorded to this single species is justified by a combination of characters that is unique among Pergidae. In particular the strongly emarginated propodeum, the petiolate anal cell of the fore



**Fig. 10:** Male of *Styracotechys dicelysma* on its host plant.

wing, and the exceptionally long tibial spurs stand out. In addition, the female has a deeply emarginate sawsheath and the male, not known to Benson when he described the species, is characterized by bipectinate antennae (Fig. 10), which occurs among the Pergidae only in the subfamily Pterygophorinae.

Females place their eggs into the midvein on the underside of young leaves (Fig. 11) at the tip of a shoot, often while the leaf is still folded longitudinally. The forked apex of the ovipositor allows the female to fix the tip of the ovipositor in place on the midvein whilst a hole for insertion of the egg is cut into the vein (Fig. 11). In the laboratory, females laid several eggs into a single leaf of the host plant, a behaviour that also occurs in nature. Hatchling larvae start to

feed on young leaves and subsequently move to older leaves as they grow.

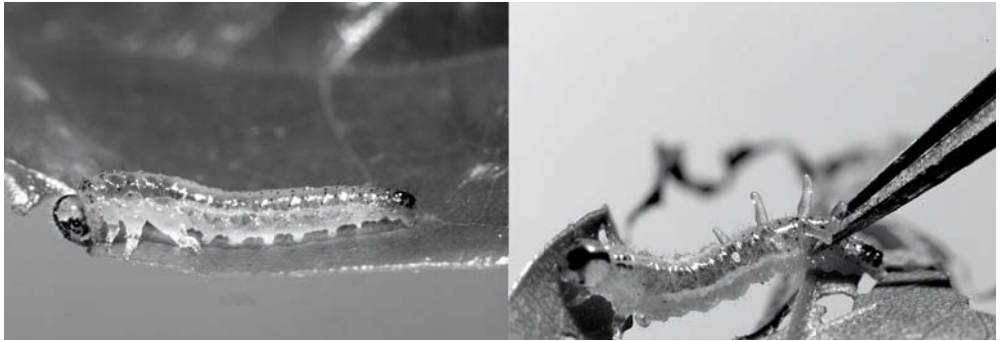
The larvae of *S. dicelysma* are whitish and somewhat transparent and the dark gut contents are clearly visible. The colour of the head is variable, ranging from whitish with a broad brown medial stripe and brown on the head below the level of the eyes (Fig. 12) to almost completely dark brown. The body is covered with long dark setae with brown bases. The apical segment has an irregular dark fleck dorsally (Fig. 12), and the first thoracic segment usually has several dark spots dorsally. The larvae feed on the leaf margin and when disturbed they extrude finger-like protrusions (Fig. 12). To our knowledge these protuberances are unique among sawfly larvae. They possibly serve a defensive purpose.

## Discussion

The serious problems connected with collecting, rearing, and maintaining pergid sawflies in the laboratory for experimental purposes have been circumvented to some extent in this study by the selection of species that are relatively more amenable to laboratory manipulation than other pergids, and this could be done only because of the extensive field experience available to us through J. G. Furthermore, techniques used for sawfly studies in the northern hemisphere were adopted to suit Australian pergid sawflies. Overall, we have presented a stronger sound basis for pergid investigations than has so far been available. Further develop-



**Fig. 11:** *Styracotechys dicelysma*. Left: Female ovipositing in the midvein of a young leaf of *Austrosteenisia blackii* (inset shows the forked ovipositor in dorsal view); right: egg inserted into the midvein of the leaf.



**Fig. 12:** Larva of *Styrocotechys dicelysma*. Left: full grown larva feeding on the leaf margin of the host plant, *Austrosteenisia blackii*; right: larva extruding finger-like projections when disturbed, in this case by gentle squeezing with a pair of forceps.

ments will help considerably in extending our understanding of this group.

The results and conclusions from our pergid studies are discussed below in the context of the current general perceptions about the evolution and diversification of the Symphyta, and in particular the Pergidae, to show that the southern radiation within this group has contributed more substantially to the current diversity of the Symphyta than previously understood.

#### Long distance attraction and specific-mate recognition

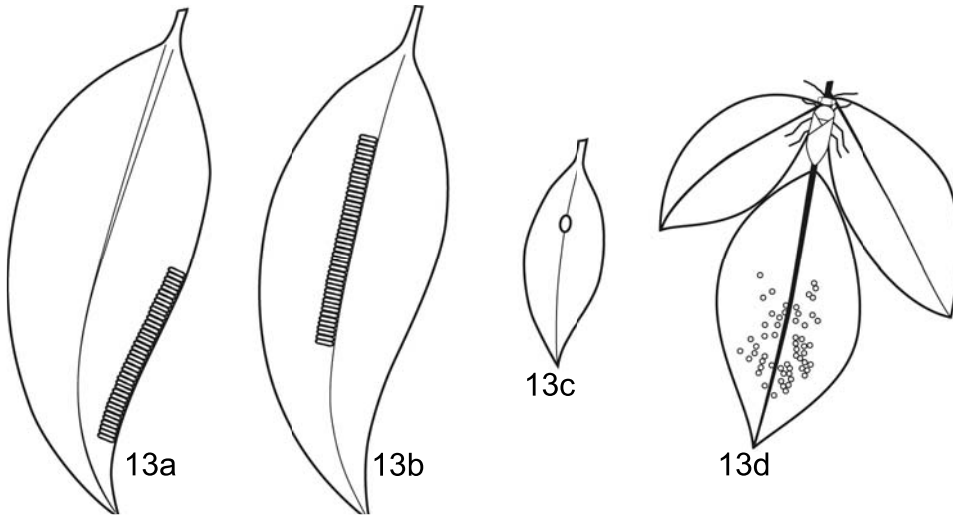
At least one species of pergid sawflies (*L. analis*) uses pheromones as long distance sex attractants (Table 2). The virgin females (but not mated ones) attract males in the field. Similarly, close-up recognition of a potential mate is mediated by chemical components of the *L. analis* female cuticle (Table 4). Behavioural observations show that acoustic and visual cues are of only minor importance, if they have any significance at all. The high numbers of sensilla on the greatly enlarged surface area of the male antennae (Fig. 6) and the difficulty to isolate even trace amounts of the pheromone indicate that only nano quantities are used for the long distance communication of *L. analis*. The sensillar equipment of the antennae differs from that found in the diprionid *Neodiprion sertifer* (cf. Hallberg 1979). There are no sex specific differences in *L. analis*, with males and females having the same types of sensilla (Fig. 6), although there are great differences in the numbers of sensilla between the sexes.

#### Mating behaviour

The mating behaviour for *L. analis* is similar to most strophandrious sawflies studied in this respect (Benson 1950, pers. obs.). After long distance attraction by the female the mating is initiated by the male and the subsequent behavioural sequence can be divided into several steps (see Results, section I c). For some pergids, in particular species of the Perginae, there are only very few observations of mating, which has led authors to suggest that other modes of reproduction predominate within the subfamily (Carne 1962, Macdonald & Ohmart 1993). However, there is no clear evidence that amphitokous parthenogenesis occurs within the Perginae (or in any other pergid subfamily) or is even the prevalent mode of reproduction in this subfamily.

#### Oviposition

The three species investigated have three different modes of oviposition. *Lophyrotoma analis* places its egg in the leaf margin (Figs 7 and 13a). Unlike most other species of the Pterygophorinae which place their eggs in a row, *L. analis* lays eggs singly. The young larvae often feed in aggregations and only later instars are solitary. In Perginae the eggpod consists of a row of eggs and is placed along the midrib of the plant leaf (Fig. 13b). The female stands on the leaf surface and deposits the egg through the midrib and into the leaf. The young larvae assemble into a tight circular position where either the heads or ends of the abdomen are juxtaposed at the periphery, with the remain-



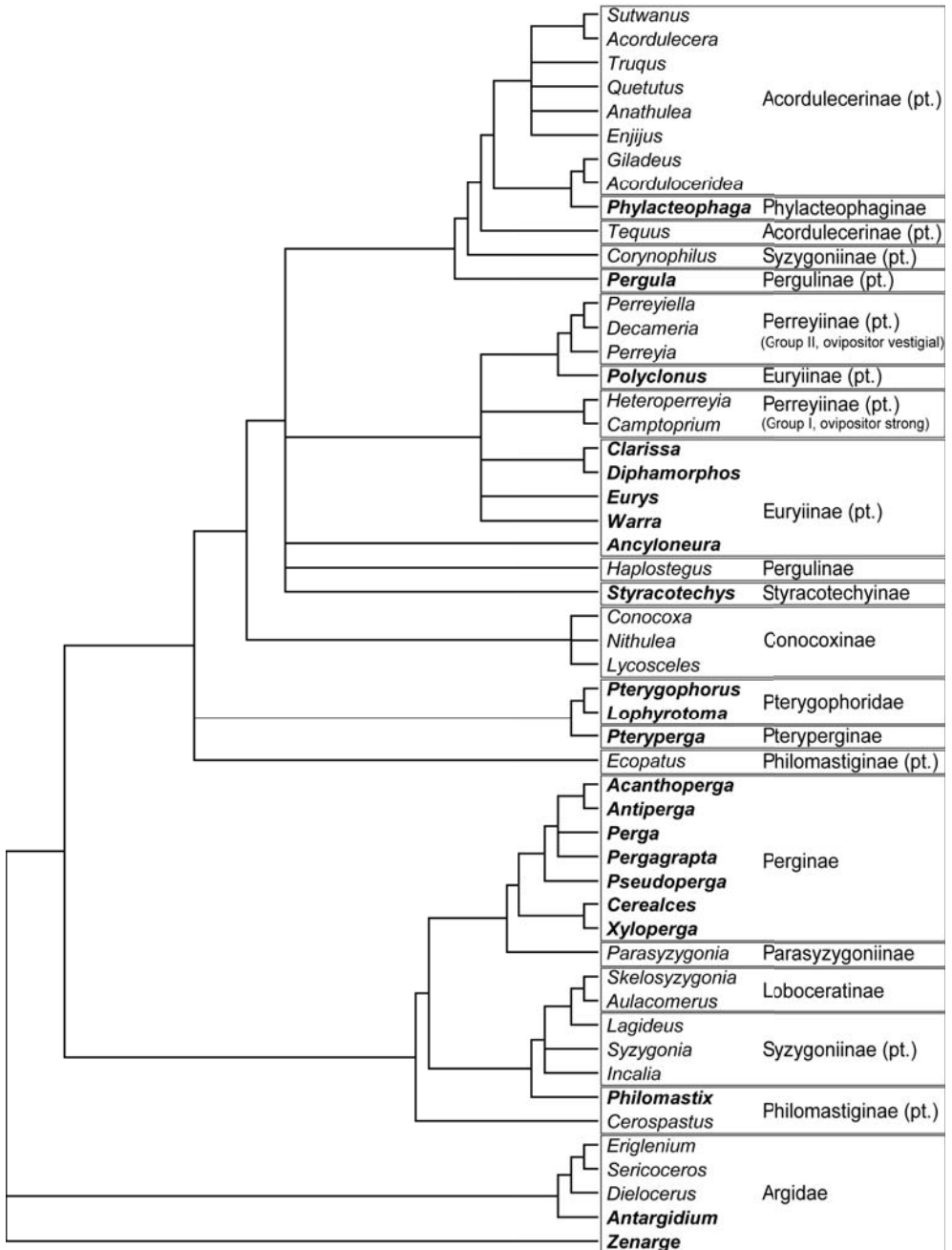
**Fig. 13:** Schematic representation of the major oviposition characteristics of Australian pergid sawflies: a) Pterygophorinae (eggpod along leaf margin, b) Perginae (eggpod along midrib of leaf), c) Styracotechyinae (single egg in midrib), d) Philomastiginae (scattered eggs on leaf underside with guarding female above, modified after Macdonald & Ohmart 1993).

ing larvae at the centre, a behaviour referred to as cycloaexy (Weinstein 1989, Jolivet et al. 1990). Females of *Styracotechys dicelysma* place a single egg into the midrib of the leaf (Figs 11 and 13c). The larvae feed solitarily at the leaf margin (Fig. 12). These species represent the major types of oviposition in Australian pergid sawflies. Females of the three known Australian species of *Philomastix* scatter their eggs on the underside of the leaf by cutting a slit through the upper surface of the leaf pushing the egg through the hole, and attaching the stalk of the egg to the leaf (Fig. 13 d). Females of all the three known *Philomastix* species then guard their eggs and feeding larvae (Leask 1944, Macdonald & Ohmart 1993).

In summary, the oviposition pattern described for each subfamily of pergids appears to be characteristic for each subfamily, but additional observations are needed. Such a diversity of oviposition types is similar in extent to the diversity of oviposition behaviours across the northern symphytan families, although in these groups eggs are usually deposited singly and embedded in the tissues of their host plants (Benson 1952).

#### Host associations, Gondwana, and the diversification of pergid sawflies

The pergids investigated generally show close associations with myrtaceous plants, their predominant hosts (Table 1). Specific morphological, chemical, and behavioural adaptations, often combined with the use of a narrow range of host plants, probably facilitated their diversification in Australia. This diversification, across and within subfamilies, seems to have been associated with the origins and diversification of the Myrtaceae, especially the genus *Eucalyptus*. This plant diversification correlates, in turn, with the geological upheaval that led to the Australian separation from South America and with the episodic alternation of arid conditions (when *Eucalyptus* would have been widespread) and mesic conditions (when rainforest predominated) across Australia. The diversity of pergids is currently interpreted as being intrafamilial, but is more likely to resemble the diversification achieved by the other Symphyta at family level. In other words, the southern diversity should be seen as more akin to the radiation that took place in the northern Symphyta, as described below.



**Fig. 14:** Preliminary phylogeny of the family Pergidae. Genera occurring in Australasia are indicated by bold face. The data set comprised 47 pergid and 5 outgroup genera (Argidae) and the analysis is based on 56 morphological characters, 54 of them parsimony-informative. The data were analysed with PAUP\* vers. 4b10 (Swofford 2002) using the heuristic search algorithm with 100 random addition sequences, TBR branch swapping, and with all characters unordered and with equal weights. The cladogram shows the strict consensus tree of 644 most parsimonious trees.

The main adaptive radiation of Symphyta is generally considered to have occurred in temperate climates of the Northern Hemisphere, because (i) the groups considered as the most ancestral occur in these regions, (ii) these groups are mostly associated with more primitive host plants, and (iii) there are more families there (Benson 1952). This view has been developed without considering two aspects of the extensive diversification of the Pergidae in the southern hemisphere. The first and more obvious aspect is the diversification within some Australian subfamilies, which apparently took place in association with the break-up period of Gondwana, the time when the radiation of the major groups of modern Myrtaceae (e.g. *Eucalyptus*, Ladiges 1997) took place. Preliminary results of studies examining phylogenetic relationships within the Pergidae (Fig. 14) suggest that the major lineages arose before the break-up of Gondwana, but after Africa had separated from Gondwana. This is supported by the fact that the myrtaceous flora of southern Africa is only poorly developed (Johnson & Briggs 1981) and that it contains neither eucalypts nor pergids. Eucalypts apparently evolved as a consequence of the increasingly dry conditions during the Tertiary and the associated contraction of rainforests that had previously covered most of Australia (Kemp 1981). Also, three of the 14 pergid subfamilies (Pergulinae, Perreyiinae, and Philomastiginae) occur both in Australasia and South America. A preliminary analysis of the phylogenetic relationships among pergid taxa (Fig. 14) shows the Australasian genera are scattered among Nearctic genera, which suggests that the major groupings existed before the separation of Gondwana.

The second aspect is that within the family Pergidae there is a level of morphological diversity across the subfamilies that is equivalent to the diversity across the remaining symphytan families. In other words, the extent of the diversification that has taken place in the Gondwana Symphyta is hidden by the higher taxa not being pegged at the family level. The "northern" families do not constitute a monophyletic lineage, whereas the Pergidae have repeatedly been confirmed to be monophyletic. Most recent analyses (Schulmeister 2003a, b) support this notion, and they show the families Argidae + Pergidae constitute a well supported monophyletic clade with the Cimbicidae in a sister-group relationship (e.g. Ronquist et al. 1999, Vilhelmsen 1997, 2001, Schulmeister

2003 a, b). Indeed, the Argidae + Pergidae clade is the best supported lineage within the Tenthredinoidea. However, all analyses have been based on only a relatively small number of species with representatives of only a few of the pergid subfamilies. Thus these analyses cannot reveal just how diverse the pergids may be, and a re-examination with a larger sample of taxa would be desirable. Even if the pergids are monophyletic, the extent of their diversification must still be considered for what it represents in terms of adaptive radiation within the Symphyta.

### Understanding Australian pergid diversity – an outlook

Within the Australian Pergidae there is a wide diversity of specialisations that have evolved in response to the challenges imposed by the local flora and abiotic environment (Macdonald & Ohmart 1993, Schmidt et al. 2000, this study). To understand host plant associations it is necessary to resolve phylogenetic relationships to determine how host relationships of pergid subfamilies were constrained. Only then will it be possible to address the question of how these sawflies shifted to myrtaceous host plants, which are unusually rich in essential oils (Boland et al. 1991). However, even basic biological data are not available for most species and only a few pergids such as *Perga affinis* have been subject to a comprehensive study (Carne 1962). Furthermore, the family contains several groups of taxonomically unresolved species complexes which need to be clarified (Macdonald & Ohmart 1993, Benson 1939, this study). Our present study addresses questions related to the sexual communication and host plant relationships of representatives of several pergid subfamilies. The challenge provided by this particular group of insects is to deal with a wide array of life history traits that require the application of a diversity of appropriate techniques. Apart from taxonomic problems, which are particularly prevalent in the major subfamilies, there is still a considerable lack of basic behavioural and ecological data. The availability of this basic knowledge is a prerequisite for studies trying to unravel the interplay between major geological and climatic changes, the biological properties of herbivores and their hosts and the diversification within a radiating lineage of phytophagous insects.

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## Abstract

**Sexual Communication and Host Plant Associations of Australian Pergid Sawflies (Hymenoptera: Symphyta: Pergidae).** The sexual communication and host plant relationships of three Australian species of the sawfly family Pergidae are investigated. *Lophyrotoma analis* (Costa) and *Pergagraptia polita* (Leach) are members of two major pergid subfamilies, Pterygophorinae and Perginae, respectively, whereas *Styracotechys dicelysma* Benson is the only known species of the subfamily Styracotechyinae. Females of *L. analis* use pheromones as long distance sex attractants. Chemical components of the female cuticle are involved in the recognition of a potential mate, whereas no significance could be assigned to acoustic and visual cues. The mating and oviposition behaviour of *L. analis* is described and illustrated. Unlike most other species of the subfamily Perginae, *P. polita* apparently is a polyphagous species that is associated with several myrtaceous host plants, including eucalypts and paperbarks (*Melaleuca* spp.). Despite the absence of noticeable morphological, behavioural or genetic differences among host-associated populations the possibility that *P. polita* represents a complex of cryptic species cannot be excluded. A comprehensive list with currently known host plants, including several new host records, is presented for species of the Perginae. The host plant relationships of *S. dicelysma* are described and illustrated for the first time. The diversification of the Pergidae is discussed in relation to geological and climatic events that led to the break-up of the Gondwanan super-continent and the origins and diversification of the major host plant family Myrtaceae.

## Zusammenfassung

**Sexuelle Kommunikation und Wirtspflanzenbeziehungen australischer Blattwespen der Familie Pergidae (Hymenoptera: Symphyta: Pergidae).** Die sexuelle Kommunikation und die Wirtspflanzenbeziehungen von drei australischen Arten der Blattwespenfamilie Pergidae wurden untersucht. *Lophyrotoma analis* (Costa) und *Pergagraptia polita* (Leach) gehören zu zwei der größten Pergiden-Unterfamilien, Pterygophorinae und Perginae. *Styracotechys dicelysma* Benson ist die einzige bekannte Art der Unterfamilie Styracotechyinae. Weibchen von *L. analis* produzieren Pheromone zur Anlockung der Männchen über größere Entfernungen. Bei der Erkennung eines potentiellen Fortpflanzungspartners sind chemische Komponenten der Kutikula beteiligt. Demgegenüber spielen optische und akustische Signale offenbar keine Rolle. Das Fortpflanzungs- und Eiablageverhalten von *L. analis* wird beschrieben und illustriert. Im Gegensatz zu anderen Arten der Perginae ist *P. polita* offenbar eine polyphage Art die mit mehreren Arten der Pflanzenfamilie Myrtaceae assoziiert ist, einschließlich *Eucalyptus*- und *Melaleuca*-Arten. Trotz des Fehlens wahrnehmbarer morphologischer, ethologischer oder genetischer Unterschiede kann nicht ausgeschlossen werden, dass es sich bei den an verschiedenen Wirtspflanzen lebenden Populationen um einen Komplex kryptischer Arten handelt. Für die Perginae wird eine umfassende Liste mit allen bekannten Wirtspflanzen gegeben. Die Wirtspflanzenbeziehungen von *S. dicelysma* werden erstmals beschrieben und illustriert. Die Diversifizierung der Pergiden im Zusammenhang mit geologischen und klimatischen Veränderungen der Umwelt, die zum Aufbrechen des Superkontinents Gondwana führten, sowie der Ursprung und die Diversifizierung der Myrtaceae werden diskutiert.

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**Fig. 5:** Mating of *Lophyrotoma analis*, a: male aligns to female, b: male attaches apex of abdomen to the female abdomen, c: mating position.



**Fig. 7.** Female of *Lophyrotoma analis* Costa ovipositing on swamp dock (*Rumex brownii*).



**Fig. 8.** Full grown larva of *Lophyrotoma analis* Costa feeding on dock (*Rumex brownii*).