The Attraction of Flowers of *Bulbophyllum* (Section *Sestochilus*) to *Bactrocera* Fruit Flies (Diptera: Tephritidae)

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SUMMARY. Observations were made on fruit flies of the genus *Bactrocera* Macquart (Diptera: Tephritidae) on *Bulbophyllum elevatopunctatum* J.J. Sm., *B. patens* King ex Hook. f. and *B. praetervisum* J.J. Verm. (section *Sestochilus*), in cultivation. The pollination ecology of some species in section *Sestochilus*, particularly those visited by *Bactrocera* flies, is discussed.

Introduction

The role of *Bactrocera* (formerly known as *Dacus*) fruit flies in orchid pollination is now attracting international attention in conservation biodiversity despite the fact that many of those fruit fly species have long been known to be major pests of fruit and vegetables (Tan, 1993 & 2009). Species of *Bulbophyllum* section *Sestochilus* are probably one of the largest groups within the genus that attract *Bactrocera* fruit flies as their pollinators.

A large number of species in section *Sestochilus*, particularly those emitting a fruity scent, are now known to attract fruit flies of the genus *Bactrocera* as their pollinators, e.g., *Bulbophyllum cheiri* (Tan et al., 2002; Nishida et al., 2004), *B. patens* (Tan & Nishida, 2000), *B. vinaceum* (Tan et al., 2006), *B. baileyi* (Tan & Nishida, 2007) and *B. apertum* (Tan & Nishida, 2005). Table 1 provides an overview of *Bulbophyllum* orchids visited by *Bactrocera* flies, including those that we observed.

These *Bulbophyllum* species produce floral scents that primarily contain methyl eugenol (ME), raspberry ketone (RK) or zingerone that attracts male *Bactrocera* flies as pollinators. For example, flowers of *Bulbophyllum cheiri* produce methyl eugenol (ME) that attracts male ME-sensitive fruit flies such as *Bactrocera dorsalis* sensu lato (thought not to be distinct from *B. papayae* that is also known to occur naturally in Peninsular Malaysia), *B. umbrosa* and *B. carambolae* (Tan et al., 2002). Whereas flowers of *Bulbophyllum apertum* produce raspberry ketone (RK) that attracts RK-sensitive fruit fly species such as *Bactrocera albistrigata*, *B. caudata*, *B. cucurbitae* and *B. tau* (Tan & Nishida, 2005). *Bulbophyllum patens*, on the other hand, releases floral zingerone that attracts both ME-sensitive and RK-sensitive fruit flies (Tan &

Table 1. *Bulbophyllum* species in section *Sestochilus* known to be visited by *Bactrocera* flies.

Species	**Observer**
B. apertum	Tan & Nishida (2005)
B. ecornutum sensu Vermeulen *1991*	Tan (2009)
B. baileyi	Smythe (1969); Christensen (1994); Tan & Nishida (2007);
	Clarke et al. (2002)
B. cheiri	Tan (1993); Tan et al. (2002); Nishida et al.(2004)
* *B. elevatopunctatum*	Jutta et al. (2008)
B. gerlandianum (syn. *B. emiliorum*)	Tan (2009)
B. gjellerupii	Tan (2009); Howcroft (1983)
B. hahlianum	Howcroft c/f Tan (2009)
B. macranthoides (syn. *B. tollenoniferum*)	Tan (2009); Clarke et al. (2002)
B. macranthum	Ridley (1890); Comber (1990 & 2001); Tan (2009)
* *B. patens*	Tan & Nishida (2000)
* *B. praetervisum*	Tan (2009)
B. sinapis	Howcroft c/f Tan (2009)
B. vinaceum	Tan et al. (2006)

* Observed by the authors

Nishida, 2000). The lips of these species have the highest concentration of these chemical compounds which explains why the visiting insects probe the lip followed by licking, resulting in triggering of the lip mechanism.

Tan (2009) showed that there are mutual reproductive benefits between the *Bulbophyllum* orchid and the *Bactrocera* fruit fly. For example, male *Bactrocera* flies that visit a flower or flowers of *Bulbophyllum cheiri* will be rewarded with methyl-eugenol that helps to boost their pheromone and defence systems (Tan et al., 2002). In return, the pollinarium is dislodged by the visiting *Bactrocera* fly and is transferred to another flower (Tan & Nishida, 2000) resulting in fertilisation. The distinction between visitor and pollinator is not difficult considering that the pollinarium is large enough to be seen by the observer either on the pollinator or that the pollinarium is absent from the flower immediately after visitation (Dressler, 1981). The capability of fruit flies in dislodging the pollinarium from *Bulbophyllum* flowers strongly suggests that they are also able to deposit the pollinarium on to the stigma.

Bactrocera flies are primarily attracted to the floral scents emitted by these bulbophyllums. When a fully open flower of *Bulbophyllum patens* was totally covered with thin black netting, it still attracted male flies of *Bactrocera caudata*, *B. cucurbitae*, *B. papayae* and *B. tau* (Tan & Nishida, 2000). It is evident that floral scent is the primary cue in long-distance attraction of the flies to the orchids. Visual cues are thought to be less important, but the mechanism involving a combination of both these cues is believed to function in attracting flies (Christensen, 1994) as the flies engage in active foraging behaviour.

The outcome of these studies has provided valuable background information allowing us to better understand the relationship between *Bulbophyllum* and *Bactrocera*. It has demonstrated that some of these species of *Bulbophyllum* are dependent on particular groups of flies for pollination. This raises some concern especially if *Bactrocera* species are considered as agricultural pests (Merritt et al., 2003; Tan, 1993 & 2009) and extensive eradication measures are taken (Tan, 2009). Koopowitz (2001) pointed out that if a species of orchid is dependent on a single pollinator and if that pollinator becomes extinct, the orchid would also become extinct. This is a potential problem for many orchid species that rely on invertebrates as pollinators if pollinator numbers decline as a result of widespread global use of chemical pesticides (Koopowitz et al., 2003). It is important, therefore, to ensure that the co-existence between *Bulbophyllum* and *Bactrocera* is maintained and conserved (Tan, 1993).

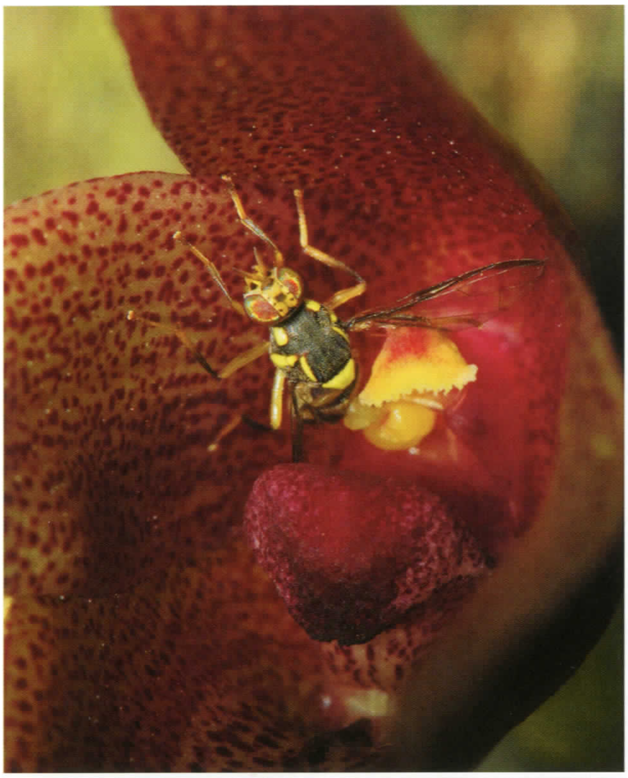
Fig. 1. Male fruit flies, *Bactrocera dorsalis* (left) and *B. umbrosa* (right) visiting the unopened flower bud of *Bulbophyllum elevatopunctatum*. Photo: P.T. Ong.

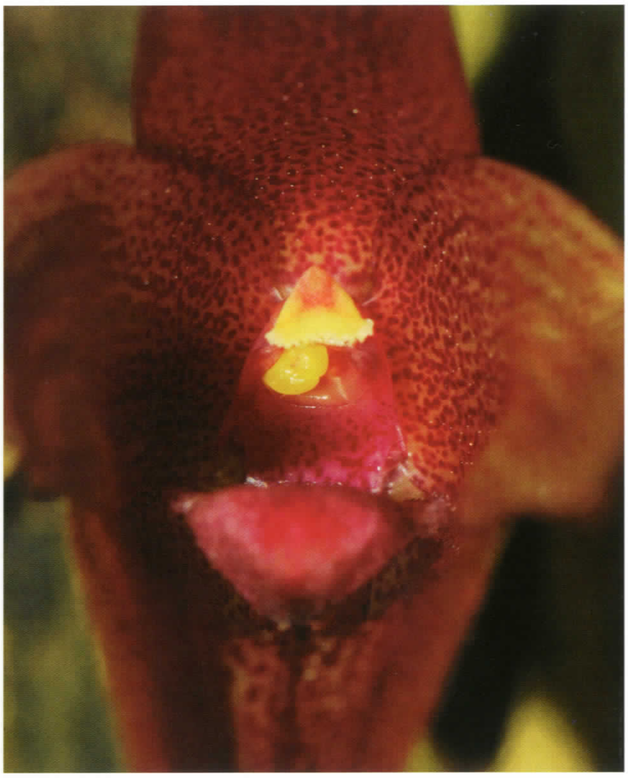
Observations of Bactrocera fly visitation to Bulbophyllum flowers in Peninsular Malaysia

Bulbophyllum elevatopunctatum J.J. Sm. ***Figs. 1–9.***

Bulbophyllum elevatopunctatum was recently reported to occur in Peninsular Malaysia (Jutta et al., 2008). In cultivation in Malaysia this species flowers frequently throughout the year. Its flowers emit a 'fruity' scent that never fails to attract *Bactrocera* flies. Jutta et al. (2008) noted that an unknown species of *Bactrocera* visited the flower as soon as the bud opened. In our observations, male flies of *Bactrocera dorsalis* and *B. umbrosa* were seen visiting the flower bud of *Bulbophyllum elevatopunctatum* just prior to opening (Fig. 1), and proceeded to probe the opening of the sepals. As soon as the flower opened, the flies were seen to probe and later lick the sepals, petals and finally the lip. As the *Bactrocera dorsalis* fly moved further on to the lip, the equilibrium was disturbed thus flicking the fly towards the column where the pollinarium was secured to the fly's thorax by the sticky viscidium. As the fly backs out from the lip, the pollinarium was removed (Figs. 2–5). This was observed on more than six occasions on different plants. In every case, all of the flies removed the pollinarium within fifteen to twenty minutes of visiting the flower. Once, a *Bactrocera dorsalis* fly that was carrying a pollinarium was seen to have become stuck to the column. After many hours of struggling, the fly finally managed to free itself (Figs. 6–7) from the column leaving the pollinia on the stigma (Fig. 8). It is interesting to note that this fly had the pollinarium attached to its abdomen instead of its thorax as it should be (see Figs. 4 & 5). This strongly suggests that the pollinarium might not belong to *Bulbophyllum elevatopunctatum*. The *Bactrocera dorsalis* flies that visit the flower as soon as it opens between 7 am and 8 am are usually the ones that remove the pollinarium. Usually, by mid-morning or afternoon, more flies were observed visiting the flower (Fig. 9) but by then, the pollinarium would have already been removed. Larger more aggressive flies chased off the smaller ones that tried to probe the flower parts. The probing and licking behaviour (feeding patterns) displayed by the flies suggests that the pollinator reward may be compounds released by the flower. Besides *Bactrocera dorsalis*, *B. umbrosa* was also seen trying to visit the flowers on

Figs. 2–5. Male fruit fly, *Bactrocera dorsalis* removing the pollinarium from the flower of *Bulbophyllum elevatopunctatum*. Photos: P.T. Ong.





Figs. 6–8. Male fruit fly, *Bactrocera dorsalis* struggling to free itself from the column after depositing the pollinia on to the stigma of *Bulbophyllum elevatopunctatum*. Photos: P.T. Ong.

two occasions. This fly, however, was unable to get close to the flower due to the presence of the more aggressive *B. dorsalis*.

Bulbophyllum patens King ex Hook. f. ***Fig. 10.***

A yellow form of *Bulbophyllum patens* that flowered several times between 2007 and 2009 was observed to be visited by male *Bactrocera dorsalis* flies (Fig. 10). Unfortunately, in all the observations, the pollinarium had already been removed by earlier visiting flies. The later visiting flies were seen probing the sepals and petals of the flower in a similar way to that described for fruit flies visiting *B. elevatopunctatum*.

Bulbophyllum praetervisum J.J. Verm. ***Figs. 11–13.***

We observed flowers of *Bulbophyllum praetervisum* being visited by *Bactrocera dorsalis* on several occasions. In October 2009, *Bactrocera caudata* and *B. tau* were also seen visiting the flower at the same time as *B. dorsalis*. On another occasion in September 2010, *Bactrocera tau* and *B. hochii* were seen visiting the flower. It is interesting to note that *Bactrocera hochii* (Fig. 11) is not considered to be a pest species. This is probably the first record of this RK-sensitive fly

Fig. 9. *Bulbophyllum elevatopunctatum* flower visited by many *Bactrocera dorsalis* flies. Photo: P.T. Ong.

visiting *Bulbophyllum praetervisum*. On a separate occasion, also in September 2010, a *Bactrocera cucurbitae* was seen stuck to the column of *Bulbophyllum praetervisum* unable to free itself. The male flies of all the species of *Bactrocera* were observed to probe the sepals and petals followed by licking behaviour. During the course of probing the flower parts, usually the lateral sepals, they tended to slip from the vertically positioned lateral sepals of the non-resupinate flowers of *Bulbophyllum praetervisum*. This resulted in the fly clinging to either the margin of the lateral sepal or the lip depending which was nearest. If the fly clinged to the lip (Fig. 12), it was immediately catapulted against the column where it dislodged the pollinarium from the anther. It is almost certain that if a fly succeeds in removing the pollinarium, it will be attached to the fly's abdomen instead of on the thorax, as in *Bulbophyllum elevatopunctatum* based on our observations of the position of flies that were stuck to the column. Observations to date also suggest that the lip of

Fig. 10. A male *Bactrocera* fruit fly probing the lip of *Bulbophyllum patens*. Photo: A.K.W. Hee.

Fig. 11. *Bactrocera hochii* (bottom left) and *B. tau* (top) probing the sepals of *Bulbophyllum praetervisum*. Photo: P.T. Ong.

Bulbophyllum praetervisum may not contain the highest concentration of floral chemical compounds (although this is yet to be proven) which the visiting flies constantly probe and lick on the sepals and petals (in particular the lateral sepals) rather than on the lip. The lip only serves to throw the fly against the column if the fly slips down from the lateral sepals and grabs hold of it. Other male *Bactrocera* flies that engage in this behaviour also experience the same fate as *B. cucurbitae* (Fig. 13). This scenario is almost identical to that observed by Ridley (1890) in *Bulbophyllum macranthum*. This is not surprising as the two species are almost identical in their pollination mechanism.

Discussion

Although pollination mechanisms in *Bulbophyllum* are generally well-known, information on direct observations on the fruit fly–*Bulbophyllum* flower interaction remains scarce. Clarke et al. (2002), for example, argued that Tan & Nishida (2000) had not presented direct evidence of the fruit fly pollinating the *Bulbophyllum*, although they provided ecological evidence of the fruit fly–*Bulbophyllum* relationship as mediated by semiochemicals (chemicals that induce behavioural changes). However, fruit flies have recently been observed to directly remove the pollinarium of certain *Bulbophyllum* species such as *B. apertum* (Tan & Nishida, 2005), *B. baileyi* (Tan & Nishida, 2007), *B. cheiri* (Tan, 1993; Tan et al., 2002) and *B. vinaceum* (Tan et al., 2006).

Van der Cingel (2001) summarised the research methodology for pollination ecological studies in which he listed eight points that must be followed: 1, vector transferring pollen from the anther must be shown; 2, vector transporting pollen must be shown; 3, vector transferring pollen to the stigma must be shown; 4, vector that deposited the pollen must effect fertilisation; 5, pollinating vector must be shown to be able to perceive and use the flower advertisement by visual and olfactory means; 6, vector must be shown to be consuming or using the flower reward as an essential part of the pollination process; 7, the result of the pollination process must be shown as a relative

Fig. 12. *Bactrocera dorsalis* fly clinging on to the lip of *Bulbophyllum praetervisum* after slipping from the lateral sepals. Photo: P.T. Ong.

Fig. 13. Close-up of a *Bactrocera cucurbitae* fly which has become stuck to the column of *Bulbophyllum praetervisum*. Photo: P.T. Ong.

contribution to the next generation; 8, different vectors involved in pollination must be shown to be interrelated.

This methodology may seem impossible to demonstrate fully in a single step. The researcher will need to start with direct observation and progress to more sophisticated analytical studies employed in disciplines such as chemical ecology to be able to fulfil these requirements in order to understand the pollination of these orchids. We, therefore, are interested in direct observation and presenting visual evidence of the fruit fly visiting and pollinating the *Bulbophyllum*. We believe that results from our method of direct observation will complement existing published information on this fascinating fruit fly–*Bulbophyllum* relationship.

Further investigation of vectors, particularly *Bactrocera* flies, is required in order to broaden our knowledge of the interrelationship between the many *Bulbophyllum* species and fruit flies. Observation of fly visits to *Bulbophyllum* in the natural habitat is most useful but may not always be practical; some *Bulbophyllum* species are not easily found and, if they are located, may not be in bloom. Thus, a well-maintained *ex-situ* collection of plants is highly desirable to enable such observations and studies to be carried out. Such observations may provide clues to the type of fruit fly species in the area known to harbour endemic fly populations that visit a particular species of *Bulbophyllum* in the wild. Finally, it is hoped that this paper will serve as a catalyst for researchers and orchid enthusiasts to pay increased attention to this intriguing interaction between orchids and their insect visitors, particularly in light of conserving our priceless yet fragile natural heritage — the *Bulbophyllum* orchids.

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