Biology of the Two Parasitoid Wasps, *Atanycolus initiator*
(FABRICIUS) and *Spathius brevicaudis* RATZEBURG
(Hymenoptera: Braconidae), on Subcortical Beetles
in Japanese Pine Trees

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Life histories, morphological characteristics and host species were investigated under room conditions in *Atanycolus initiator* and *Spathius brevicaudis*, two major species of solitary wasps which parasitize subcortical beetles infesting Japanese pine trees. The duration of successive stages from egg hatching to adult emergence was about 16 days for both species. Females of both species required more time than males for development. *A. initiator* was about eight times larger in body weight and had an ovipositor 3.7 times longer than that of *S. brevicaudis*. While both wasps were polyphagous and some host species were common to them, there was a distinct discrimination in host size selection between the two parasitoid species.

Key words: *Atanycolus initiator*, parasitoid wasps, pine beetles, *Spathius brevicaudis*, subcortical beetles

INTRODUCTION

Two species of Braconid wasps, *Atanycolus initiator* (FABRICIUS) and *Spathius brevicaudis* RATZEBURG (Fig. 1), are major solitary parasitoids on subcortical beetles infesting Japanese pine trees. Both species are polyphagous, mostly parasitizing larvae of Cerambycidae, Curculionidae and Scolytidae.

A. initiator is widely distributed in the Palaearctic (SHENEFELT, 1978) and is known as an ectoparasitoid of long-horned beetles in Europe (STARÝ et al., 1988). In Japan, it was first described in 1937, following which several species of long-horned beetles and weevils have been reported as its hosts (NOBUCHI, 1980).

S. brevicaudis is distributed in Europe and is ectoparasitic on weevils and bark beetles (SHENEFELT, 1978). This report is the first description of this species in Japan. There are many species within the *Spathius* genus important for control of forest insect pests (BUSHING, 1965; NOBUCHI, 1980). *Spathius razayanus* RATZEBURG, for example, is a principal species parasitic on weevils and bark beetles in Japan. In North America, many congeners were listed as parasitoids of Scolytidae (BUSHING, 1965).

In the vicinity of Nagoya City, these two species comprise the majority of the

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Fig. 1. Female adults of the two parasitoid wasps: *Atanycolus initiator* (left) and *Spathius brevicaudis* (right).

parasitoids, and hence seem to play an important role in regulating the population levels of pest beetles. Nevertheless, there has been no intensive research on their biology.

The major aim of this paper is to clarify the biological features of these parasitoid wasps, by means of comparative studies of their life histories, morphological characteristics and host species.

MATERIALS AND METHODS

Sample trees were taken from the Nagoya University campus in Nagoya City (Plot I) and from the Aichi Branch of the Tokyo University Forest in Seto City (Plot II) situated about 20 km east of Nagoya City. Both sites are dominated by Japanese pines, *Pinus thumbergii* PARLATORE and *P. densiflora* SIEB. et ZUCC., followed by *Quercus serrata* THUNB and *Q. glauca* THUNB. In both plots, about 10% or less of living pine trees die every year mainly due to the pine wilt disease. A total of 13 sample trees of both pine species were used (Table 1).

Three sample trees (Nos. 1–3 in Table 1) died in Plot I between summer and autumn in 1987 and were subsequently infested by subcortical beetles, which were then *in situ* parasitized by the parasitoids. These sample trees were kept in an outdoor cage with 1 mm mesh in the Nagoya University campus between November 1987 and April 1988, and emerging wasps were used for oviposition experiments on other trees (Nos. 4–13) and measurements.

Ten other (living) trees were felled in Plot II. The stem of each tree was divided into 1 m logs and laid horizontally on the forest floor, thus exposed to attack by beetles. The data for the sample trees and the period of the experiments are given in detail in Table 1. About one month after the cutting, the infested sample logs were transferred to the outdoor cage, from which two 1 m log samples were brought to the laboratory

Table 1. Sample trees and period of experiment

Tree No.	Species ^a	Height (m)	No. of logs ^b	State of tree ^c	Site ^d	Date of tree felling	Period of experiment ^e
1	D	11	9	D	Plot I	Nov. 1987	Apr. 1988
2	D	6	5	D	Plot I	Nov. 1987	Apr. 1988
3	T	9	7	D	Plot I	Nov. 1987	Feb.-Apr. 1988
4	T	12	10	L	Plot II	Apr. 1988	May-June 1988
5	T	12	10	L	Plot II	May 1988	June-July 1988
6	T	12	10	L	Plot II	July 1988	Aug. 1988
7	T	10	8	L	Plot II	Aug. 1988	Sept.-Oct. 1988
8	T	10	8	L	Plot II	Sept. 1988	Oct.-Nov. 1988
9	T	10	8	L	Plot II	Oct. 1988	May-July 1989
10	T	10	8	L	Plot II	May 1989	July-Aug. 1989
11	T	10	8	L	Plot II	June 1989	Aug.-Sept. 1989
12	T	12	10	L	Plot II	Aug. 1989	Sept.-Nov. 1989
13	T	12	10	L	Plot II	Sept. 1989	Nov.-Dec. 1989

^a D: *Pinus densiflora*, T: *Pinus thumbergii*.

^b Number of logs (1 m in length) taken from each sample tree.

^c D: Dead tree, L: Living tree.

^d Plot I: Nagoya, Plot II: Seto.

^e Period of the experiment for the overwintering parasitoids under bark (Nos. 1-3) or for the lab-reared parasitoids (Nos. 4-13).

every week. They were cut to suitable length and set in glass jars (18 cm (ϕ) $\times$ 24 cm (H)), or PVC jars (25 cm (ϕ) $\times$ 60 cm (H)) for the log samples with diameters greater than 15 cm. Two female wasp individuals that had emerged from trees Nos. 1-3 were introduced into each glass jar and allowed to oviposit on the subcortical host beetles for three days. Three females were released over a period of seven days for each log sample in the PVC jar. For every treatment, both species of parasitoids were separately used and provided with subdivided logs derived from the same sample log. This prevented the differences in size distribution and density of host beetles between the sample logs from affecting ovipositional behavior.

Female wasps used for all the experiments were thus those emerged from sample trees Nos. 1-3, and their female offspring taken from trees Nos. 4-13 and reared in the laboratory. Each female was subjected to mating with a male immediately after emergence, a few days after which each female was used for the first oviposition experiment. One female wasp was repeatedly used for the experiments 3-5 times.

After each oviposition experiment, the parasitized hosts were individually moved from the logs to a styrene case with moisturized cotton. The developmental periods in each stage of the two parasitoid species were determined under room conditions (22-35.5°C) between April and September in 1989, and under a constant temperature (27°C) between October and December in 1989. Body length, head width and ovipositor length of the emerging adults, including those from trees Nos. 1-3, were measured with a micrometer, following which fresh body weight was weighed. The emerging adults were reared on dilute honey to detect longevity and to provide for the oviposition experiments.

RESULTS

Life histories of the two parasitoids

Females of *A. initiator* and *S. brevicaudis* searched for subcortical hosts, walking over and tapping their antennae on the bark surface of infested tree logs. Bark crevices or exit holes excavated by weevils were particularly subjected to intensive search. After several antennal probes followed by a few additional ovipositor insertions (probably to detect more accurately the location and size of host larvae), female wasps penetrated their ovipositors through bark and laid single eggs on or near each target. Hosts were "anesthetized" with the venom injected by the wasp at the time of oviposition. The time required for depositing a single egg on a host, including the ovipositor insertion into the bark, ranged from 0.5 to 3 hr, clearly longer than the time required for antennal host-searching (2–20 min).

The eggs of both parasitoid species were cylindrical with lengths of 1.1–1.7 mm (*A. initiator*) and 0.8–1.1 mm (*S. brevicaudis*) respectively. Eggs were equipped with a distinctive stalk that may be of some use for successful contact with a host. The egg stalk of *A. initiator* was much longer than that of *S. brevicaudis*, almost equalling the egg length.

The hatched larva, 1.0–1.3 mm (*A. initiator*) or 0.6–0.7 mm (*S. brevicaudis*) in length, moved actively. They were attached externally and fed by fixedly sucking in the host body fluids.

The number of instars was estimated in terms of the number of the exuviae stuck on the host body. Of the 21 hosts parasitized by *A. initiator*, four exuviae were observed on each of five hosts, and three exuviae on each of the others. Of the 15 hosts parasitized by *S. brevicaudis*, four exuviae were recognized on one host, three on each of seven hosts and two on each of the others. The exuviae of younger larvae, however, were so small that they could not be identified. These results imply that both species have five larval instars, which has been assumed for most of ectoparasitic parasitoids (STARÝ et al., 1988).

The full-grown larva left the host in order to spin a cocoon. Larvae of both species, particularly those of *S. brevicaudis*, had a tendency to leave the greater part of large host bodies unconsumed.

Figure 2 shows the seasonal changes in the total developmental period (in days) of parasitoid wasps (mean±SD) and those in temperature (mean with range) in the laboratory from April to September 1989. The total developmental periods of both species were almost identical, although the development of *A. initiator* was slightly faster than that of *S. brevicaudis* between May and August, and inversely correlated with increasing room temperature.

The period required for each growing stage under a constant temperature (27°C) is illustrated for *A. initiator* (30♂, 19♀) in Fig. 3(A), while that for *S. brevicaudis* is illustrated on the basis of the result from the September-cohort under room condition (23–34.5°C; Fig. 3(B)). In both species, females required more time than males from egg hatching to adult emergence ($t=3.78$, $p<0.001$ for *A. initiator*; $t=4.07$, $p<0.001$ for *S. brevicaudis*). For the period of each developmental stage, there were significant differences between both sexes for both species in the duration period from egg hatching to the end of host feeding (larva in stage I: $t=2.29$, $p<0.05$ for *A. initiator*; $t=1.70$, $p<0.05$ for *S. brevicaudis*) and in pupa ($t=3.75$, $p<0.001$ for *A. initiator*; $t=3.62$, $p<0.001$ for *S. brevicaudis*).

Fig. 2. (A) Room temperature of the laboratory each month between April and September in 1989 (mean and range). (B) Total developmental times (days) of the two parasitoid wasps (mean $\pm$ SD) for each monthly cohort. Figures near the circles indicate sample size. ●: *A. initiator*, ○: *S. brevicaudis*.

Fig. 3. Developmental times in each stage of the two parasitoids. (A) *A. initiator*: reared under a constant temperature (27°C) (●: ♂ ($n=30$), ▲: ♀ ($n=19$)). (B) *S. brevicaudis*: reared under room conditions (22.5–34.5°C) for the September-cohort in 1989 (○: ♂ ($n=22$), △: ♀ ($n=16$)). LI: duration from egg hatching to the end of host feeding, LII: from the end of host feeding to cocoon spinning, LIII: from cocoon spinning to pupation.

Fig. 4. Emergence patterns of the overwintered generation of *A. initiator* in the tree No. 1, after transfer of the logs into the laboratory at the beginning of April 1989. Arrows indicate the mean values for males (15 days) and females (16 days).

Table 2. Adult longevity of the two parasitoid wasps (days)

	<i>A. initiator</i>		<i>S. brevicaudis</i>		<i>t</i> value (A×S) ^a
	Mean±SD	Min-Max	Mean±SD	Min-Max	
Males	38.4±16.7 (n=51)	4-69	59.3±27.4 (n=34)	14-123	4.38 (p<0.001)
Females	65.0±23.3 (n=46)	24-111	78.1±36.9 (n=60)	26-172	2.07 (p<0.05)
<i>t</i> value (♂×♀) ^b	6.28 (p<0.001)		2.57 (p<0.005)		

^a Between species. ^b Between sexes.

Although the matured larva generally spins a cocoon and pupates in it, most last instar larvae reared in the laboratory entered diapause in their cocoons without pupation between November and December. Figure 4 shows the emergence pattern of the overwintered generation of *A. initiator* in the No. 1 sample tree for each sex, quantified in days after transfer of the logs into the laboratory at the beginning of April 1989. On average, most female adults emerged a few days after males did ($t=4.37$, $p<0.001$). The mating time was estimated to be 1-2 min for *A. initiator* and several sec for *S. brevicaudis*.

Under field conditions, the adults of both species were usually found from April to November, whereas most of the last instar larvae entered diapause in their cocoons after October. Judging from the result of lab-rearing, it seems likely that the two parasitoid wasps could yield more than eight generations annually in the laboratory.

Table 2 demonstrates the longevity of adults of the two parasitoids which were reared in a styrene case with dilute honey in the laboratory. The mean longevity of *S. brevicaudis* was significantly longer than *A. initiator*, and females of both species lived longer than males.

Table 3. Comparison of the sizes of adults between the two parasitoid wasps

	<i>Atanycolus initiator</i>			<i>Spathius brevicaudis</i>			<i>t</i> value (A × S)
	Mean ± SD	Min-Max		Mean ± SD	Min-Max		
Body length (mm)	♂ 5.5 ± 1.5 ♀ 8.2 ± 1.7	♂ 3.3-8.6 ♀ 4.6-11.0	(n=60) (n=31)	♂ 3.4 ± 0.7 ♀ 4.3 ± 0.9	♂ 2.2-4.7 ♀ 2.3-5.6	(n=43) (n=83)	8.28* 15.73*
<i>t</i> value (♂ × ♀)	7.59*			5.71*			
Head width (mm)	♂ 0.99 ± 0.18 ♀ 1.24 ± 0.20	♂ 0.70-1.40 ♀ 0.93-1.65	(n=35) (n=44)	♂ 0.62 ± 0.05 ♀ 0.75 ± 0.09	♂ 0.45-0.78 ♀ 0.50-0.93	(n=27) (n=81)	10.32* 18.74*
<i>t</i> value (♂ × ♀)	5.74*			7.05*			
Body weight (mg f. wt)	♂ 6.99 ± 5.30 ♀ 13.89 ± 5.37	♂ 0.70-30.00 ♀ 2.98-29.40	(n=108) (n=45)	♂ 0.89 ± 0.45 ♀ 1.98 ± 0.78	♂ 0.14-1.97 ♀ 0.30-3.89	(n=31) (n=75)	6.36* 19.03*
<i>t</i> value (♂ × ♀)	7.26*			7.24*			
Ovipositor length (mm)	11.0 ± 0.3	7.4-14.9	(n=44)	3.0 ± 0.1	1.5-4.3	(n=95)	47.99*

* $p < 0.001$.

Size characteristics of the two parasitoids

The average body sizes of the two species are given in Table 3, showing much size variability for both species. A remarkable example was found in *A. initiator* males; the largest individual had about 43 times the fresh body weight of the smallest one.

A. initiator was significantly larger than *S. brevicaudis* in every size parameter. The mean body weight of *A. initiator* was about eight times (males) or seven times (females) as heavy as *S. brevicaudis*. Large difference in mean ovipositor length between the two species (11 mm for *A. initiator* vs 3 mm for *S. brevicaudis*) should be noted.

The correlation coefficients (*r*) between the size parameters (body length, head width, body weight and ovipositor length) were significant in all combinations.

Host species

Both parasitoids were polyphagous and parasitized several species of Cerambycidae and Curculionidae. *S. brevicaudis* also parasitized Scolytidae (Table 4). The Japanese pine sawyer, *Monochamus alternatus* HOPE, was parasitized by neither of the parasitoids. In the laboratory, although some females of both species were observed to penetrate

Table 4. Host species and numbers of their individuals recorded from the sample logs

Host species	Total number ^a	<i>A. initiator</i> parasitized		<i>S. brevicaudis</i> parasitized	
		Number	Size mean $\pm$ SD ^b (Size range) (mg f.wt)	Number	Size mean $\pm$ SD ^b (Size range) (mg f.wt)
Cerambycidae					
<i>Monochamus alternatus</i>	200	0	207.23 $\pm$ 152.21 ^c (6.82—554.28)	0	
<i>Acanthocinus griseus</i>	65	24	74.18 $\pm$ 50.45 (5.21—203.14)	5	22.84 $\pm$ 14.14 (8.45—40.32)
<i>Arhopalus coreanus</i>	205	10	13.19 $\pm$ 11.80 (3.35—73.60)	15	9.39 $\pm$ 4.65 (2.62—18.27)
Curculionidae					
<i>Niphades variegatus</i>	266	85	66.54 $\pm$ 34.67 (4.73—183.02)	24	26.21 $\pm$ 21.71 (2.68—39.06)
<i>Shirahoshizo</i> spp.	2173	248	28.51 $\pm$ 14.71 (5.14—60.43)	213	18.43 $\pm$ 10.84 (1.52—43.34)
<i>Pissodes obscurus</i> or <i>P. nitidus</i>	316	2	47.12 $\pm$ 12.37 (38.37—55.87)	52	8.73 $\pm$ 9.06 (2.27—48.14)
Scolytidae					
<i>Tomicus piniperda</i>	856	0		209	6.65 $\pm$ 2.89 (1.21—11.29)
<i>Orthotomicus angulatus</i>	171	0		42	1.96 $\pm$ 0.72 (0.46—3.16)
<i>Cryphalus fulvus</i>	^d	0	0.42 $\pm$ 0.15 ^e (0.13—0.72)	0	

^a Total number of individuals of each host species in the sample trees Nos. 4–13.

^b Size of parasitized beetle larvae by each parasitoid (mg f. wt).

^c Forty-two unparasitized larvae were measured.

^d Not counted.

^e Sixty-three unparasitized larvae were measured.

Fig. 5. Size distribution (based on head capsule width) of *Shirahoshizo* spp. larvae parasitized by each parasitoid species, *A. initiator* ($n=144$) and *S. brevicaudis* ($n=197$). All data used were collected from sample trees Nos. 1–8 between April and November 1988. Arrow indicates each mean value.

their ovipositors into logs exclusively infested by *M. alternatus*, none of them actually oviposited. A number of log samples heavily infested with *Cryphalus fulvus* NIJIMA also remained untouched. *Shirahoshizo* spp., including *S. rufescens* ROELOFS, *S. insidiosus* R. and *S. pini* MORIMOTO, were the most abundant host species in all sample logs. Consequently, as much as 67% of *A. initiator* and 38% of *S. brevicaudis* emerged from these *Shirahoshizo* spp.

It is noticeable that there was large variation in size of individuals parasitized by either wasp species and that in all common host species the average size of *A. initiator* hosts was clearly larger than that of *S. brevicaudis* hosts (Table 4). Evidence was shown in terms of the size distribution (based on head capsule width) of *Shirahoshizo* spp. larvae parasitized by each parasitoid, which is based on the data collected throughout 1988 (Fig. 5). The size distribution of *Shirahoshizo* spp. parasitized by *A. initiator* was, as a whole, biased to larger host size class and had obviously narrower size-range than that used by *S. brevicaudis*.

DISCUSSION

The life histories of *A. initiator* and *S. brevicaudis* in this study were almost identical with each other and other Braconid species, e.g. *Dendrosoter protuberans* (KENNEDY, 1970) and *Coeloides brunneri* (RYAN and RUDINSKY, 1962).

Both species required a relatively long period of time to deposit an egg on each

host, which is specific to the wasps parasitic on subcortical beetles. They may detect the hosts under the bark by receiving the sound emitted by the chewing activity of the host larvae (DE LEON, 1935; RYAN and RUDINSKY, 1962) or through the perception of host-generated infrared radiation (RICHERSON and BORDEN, 1972). Other factors measuring host characteristics such as size would be evaluated by the ovipositor probes. Longer handling time could thus be largely due to these host searching and egg-depositing processes, contrasting with wasp species parasitizing exposed hosts (e.g. SATO, 1979).

As demonstrated in Table 4, there was a distinct discrimination in selecting host species between *A. initiator* and *S. brevicaudis*. The size distribution of the common host, *Shirahoshi* spp., was significantly more biased to larger size classes in *A. initiator* than in *S. brevicaudis* (Fig. 5). A possible reason to the distinctive difference in host size selection between the two parasitoids is the difference in nutritional requirements: *A. initiator* has a much larger body size than *S. brevicaudis* and therefore requires more nutrition for its development.

In this study, no scolytid beetles were parasitized by *A. initiator*. However, the largest size class of *Tomicus piniperda*, which was the most abundant scolytid in our study, was sometimes much heavier than that in the smallest size class of other host species actually parasitized by *A. initiator* (Table 4). However, larvae of *T. piniperda* were only found in the No. 9 sample tree, where they coexisted with those of much larger *N. variegatus*. *A. initiator* thus preferred relatively large hosts such as *N. variegatus*, by which more fitness would be expected. On the other hand, *S. brevicaudis* could use *T. piniperda* as its host because the body size of the wasp was small and therefore could acquire enough fitness from the bark beetle.

TOGASHI (1989) reported that *A. initiator* parasitized 2nd to 4th instar larvae of Japanese pine sawyer, *Monochamus alternatus*, in the field. *M. alternatus* was the largest larva in this study (Table 4), and seems to be suitable for successful development of both parasitoids. No female wasps, however, laid eggs on larvae of the sawyer. We cannot yet explain why *M. alternatus* was rejected here by the wasp species as their hosts, whereas accepted elsewhere.

A. initiator and *S. brevicaudis*, developing in the same pine trees, had almost the same life history and some common host species. However, they had clear differences in their body sizes and preference of host size. These results suggest that some competitive interactions could be functioning between the two parasitoid species for the same host complex in a tree. More detailed analyses are needed for clarifying the factors affecting the interactions of these two parasitoid species.

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