Parasitoid complex of overwintering cocoons of *Neodiprion huizeensis* (Hymenoptera: Diprionidae) in Guizhou, China

Complejo de parasitoides de capullos invernales de Neodiprion huizeensis (Hymenoptera: Diprionidae) en Guizhou, China

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Abstract: The conifer sawfly, *Neodiprion huizeensis* (Hymenoptera: Diprionidae), is an injurious leaf feeder of *Pinus* spp. (Pinaceae) in China. Its parasitoid complex of overwintering cocoons was investigated in Weining, Guizhou during 2012. The average parasitism rate of overwintering cocoons of *N. huizeensis* by the parasitoid complex was 34.6%. The parasitoid complex included *Drino auricapita* (Diptera: Tachinidae), ichneumonids, and *Trichomalus* sp. (Hymenoptera: Pteromalidae). The average parasitism rate of *N. huizeensis* by *D. auricapita* was 13.1%. The puparial period of *D. auricapita* average 16.4 ± 0.1 d. The female to male ratio was 1.1: 1. The ichneumonid complex included *Aptesis grandis*, *A. melana*, *A. nigricoxa*, *Delomerista indica*, *Lamachus rufiabdominalis*, *L. nigrus*, *Bathythrix* sp., *Caenocryptus* sp., *Exyston* spp., *Gelis* sp., *Goryphus* sp., and *Olesicampe* sp. The parasitism rate of *N. huizeensis* by *Trichomalus* sp. was 4.5%, and the female to male ratio was 3.7: 1. The dominant species of parasitiods was *D. auricapita* followed by *A. melana*. The emergence of overwintered adults of *N. huizeensis* had two peaks: the first from the 17th to the 23rd of February, 2012; the second from February 29th to March 15th, 2012.

Key words: Neodiprion huizeensis. Parasitoid complex. Dominant species. Superparasitism. Sex ratio.

Resumen: En China, la avispa de sierra de las coníferas *Neodiprion huizeensis* (Hymenoptera: Diprionidae) es un insecto perjudicial de las hojas de *Pinus* spp. (Pinaceae). Se estudió el complejo parasitoide de los capullos de hibernación (CPCH) en Weining, Guizhou, 2012. La tasa de parasitismo promedio del CPCH de *N. huizeensis* fue 34,6%. El complejo de parasitoide incluye *Drino auricapita* (Diptera: Tachinidae), ichneumónidos y *Trichomalus* sp. (Hypemoptera: Pteromalidae). La tasa de parasitismo promedio de *D. auricapita* en *N. huizeensis* fue 13,1%. El período promedio del pupario de *D. auricapita* fue 16,4 \pm 0,1 d. La proporción hembra y macho fue 1,1: 1. El complejo ichneumónidos incluye *Aptesis grandis, A. melana, A. nigricoxa, Delomerista indica, Lamachus rufiabdominalis, L. nigrus, Bathythrix* sp., *Caenocryptus* sp., *Exyston* spp., *Gelis* sp., *Goryphus* sp. y *Olesicampe* sp. La tasa de parasitismo de icheumónidos en *N. huizeensis* fue 17,1% mientras que la de *Trichomalus* sp. en *N. huizeensis* fue 4,5%. La proporción hembra : macho fue 3,7: 1. Las especies dominantes de parasitoides fueron *D. auricapita* y *A. melana*. La emergencia de adultos hibernados de *N. huizeensis* tuvo dos picos, el primero del 17 al 23 de febrero de 2012 y el segundo del 29 de febrero al 15 de marzo.

Palabras clave: Neodiprion huizeensis. Complejo de parasitoides. Especie dominante. Superparasitismo. Proporción de sexos.

Introduction

The conifer sawfly, *Neodiprion huizeensis* Xiao & Zhou, 1998, belongs to the genus *Neodiprion* of Diprionidae (Hymenoptera: Symphyta). This genus includes 51 described species (Taeger *et al.* 2010), of which 6 are known from China (Wei *et al.* 2006). *Neodiprion huizeensis* is a well-known pest defoliating *Pinus armandii* Franch. (Xiao *et al.* 1984, 1992) and *Pinus yunnanensis* Franch. (Pinaceae) (Xu *et al.* 1990) in Yunnan and Guizhou, South China.

Neodiprion huizeensis has two generations a year in China. Mature larvae spin cocoons in the litter under the crowns of trees and overwinter as diapausing prepupae. The research on *N. huizeensis* has been focused on biology, ecology and integrated management, especially chemical control (Xu *et al.* 1990; Xu 1998). Parasitoids of this sawfly have not as well been studied in China. One paper found that the parasitism rate of sawfly cocoons was about 10.0% (Xu *et al.* 1990). Four parasitoids, *Aptesis melana* Li & Sheng, 2013, *Aptesis nigricoxa* Li & Sheng, 2013, *Lamachus nigrus*

Li, Sheng & Sun, 2012, and *Lamachus rufiabdominalis* Li, Sheng & Sun, 2012, (Hymenoptera: Ichneumonidae), have been reported on *N. huizeensis* (Li *et al.* 2012a, 2013).

Systematic research on the parasitoid complex will be important for biological control of *N. huizeensis*. The purpose of this survey was to determine the principal species of parasitoids from overwintering cocoons of *N. huizeensis*.

Materials and methods

Field investigation and rearing conditions. Overwintering cocoons of *N. huizeensis* were collected in Weining County ($26^{\circ}54^{\circ}N$, $104^{\circ}13^{\circ}E$, elevation 2,000 to 2,400 m), Guizhou Province. The three samples (N = 2225, 2040 and 1908 cocoons, respectively) were taken on 6th to 8th January 2012. The cocoons were kept at 2 °C one week under laboratory conditions, and then they were stored individually in glass tubes (100 mm long and 15 mm in diameter) with a piece of filter paper dipped in distilled water in order to prevent desiccation, plugged with absorbent cotton, and maintained

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at 23 ± 1 °C with 60% to 70% relative humidity and 14 hours to 10 hours light and dark photoperiod.

All cocoons were checked daily for sawfly and parasitoid emergence until late July. Emerged parasitoid larvae and pupae were kept in glass tubes at the same temperature until adult emergence. After emergence of sawflies and parasitoids was complete (that is to say, there were no sawflies or parasitoids emergence), all remaining cocoons were dissected and their condition (i.e., the status of the diprionid adults and larvae, and parasitism) was recorded. Adults of *N. huizeensis* and its parasitoids were collected with intercept trap (Li *et al.* 2012b) in the field.

Identification. Tachinid parasitoids were identified by Dr. Chun-Tian Zhang (Shenyang Normal University, P. R. China), chalcid parasitoids by Dr. Stefan Schmidt (Zoologische Staatssammlung München, Germany), *Delomerista indica* Gupta, 1982, by Dr. Gavin R. Broad (Department of Entomology, the Natural History Museum, United Kingdom), *Gelis* sp. by Dr. Martin Schwarz (Eben 21, A-4202 Kirchschlag, Austria) and *N. huizeensis* by Dr. Mei-Cai Wei (Central South University of Forestry and Technology, P. R. China). All specimens have been deposited in the Insect Museum, General Station of Forest Pest Management, State Forestry Administration, P. R. China.

Statistical analysis. Parasitism rates data (*p*) were transformed by arcsin (*p*)^{1/2} in order to better fit the assumptions of normality and homogeneity of variances for ANOVA. The means were analyzed by one-way ANOVA, followed by the Ryan-Einot-Gabriel-Welsh (REGW) multiple Q test (SPSS 17.0 for Windows) at $\alpha = 0.05$ (Li *et al.* 2012c).

Results

The parasitoid complex of *N. huizeensis* included *Drino* auricapita Chao & Liang, 1998, (Diptera: Tachinidae), Aptesis grandis Sheng, 1998, A. melana Li & Sheng, 2013, A. nigricoxa Li & Sheng, 2013, Delomerista indica Gupta, 1982, Lamachus rufiabdominalis Li, Sheng & Sun, 2012, L. nigrus Li, Sheng & Sun, 2012, Bathythrix sp., Caenocryptus sp., Exyston spp., Gelis sp., Goryphus sp., Olesicampe sp. (Hymenoptera: Ichneumonidae), and Trichomalus sp. (Hymenoptera: Pteromalidae).

From the overwintering cocoons of *N. huizeensis* 1063, 964, and 1006 adult sawflies emerged and 372, 347 and 280 dead adults and larvae were discovered from dissecting cocoons (Table 1).

From the cocoons, 340, 311 and 320 adult ichneumonids emerged, and 36, 17 and 27 larvae were discovered from Drino auricapita larvae developed inside the body of N. huizeensis larvae (Fig. 1A). Most tachinid larvae made an emergence hole on the sawfly cocoons after they emerged. Five overwintering sawfly cocoons were superparasitized by D. auricapita. One cocoon included 3 larvae of D. auricapita, other four cocoons included 2 larvae of D. auricapita, respectively (Fig. 1B). 278, 288 and 189 tachinid larvae emerged from the cocoons of N. huizeensis, and 19, 26 and 11 larvae were discovered from dissected host cocoons. The parasitism rates of D. auricapita ranged from 10.5% to 15.4%, with an average of 13.1% (Table 1).

The puparial case of *D. auricapita* is completed within 24 hours after the mature larva emergence from host remains. The newly pupae are cream-colored, and turn yellowish brown in several hours, and then turn bright red to dark red. The duration of pupa was studied and the results showed that the mean ranges of duration of pupa are 16.7 ± 0.1 , 16.4 ± 0.1 and 16.2 ± 0.1 days, respectively. There were no significant differences of the duration of pupa among the collections (F = 6.92, df = 2, 658, P > 0.05). There were 243, 263 and 155 adults of tachinid emerged. The ratio of adult females to males was 1.1: 1 (Table 3).

Trichomalus sp. is a gregarious, primary ectoparasitoid of *N. huizeensis* larvae (Fig. 1C). Depending on the size of the host, 2 to 46 individuals emerged from a single cocoon, with an average of 13 individuals from a single cocoon. One cocoon was multiparasitized by *Trichomalus* sp. and the endoparasitic larva of *D. auricapita* (Fig. 1D). There were 88, 70 and 61 cocoons of *N. huizeensis* parasitized by this chalcid. The parasitism rates of *Trichomalus* sp. ranged from 3.9% to 5.3%, with an average of 4.5% (Table 1). The female to male ratio was 3.7: 1. From 49 sawflies cocoons only female chalcids emerged.

The parasitism rates of *N. huizeensis* by parasitoid complexes ranged from 32.6% to 35.8%, with an average of 34.6%.

The ichneumonids of *N. huizeensis* included *Aptesis* grandis, which emerged 231 adults. The female to male ratio was 1.2: 1. There were 537 adults of *A. melana* emerged from sawfly cocoons, and the female to male ratio was 1.7: 1. 87 adults of *Delomerista indica* emerged from sawfly cocoons, and the female to male ratio was 2.5: 1. 78 adults of *Lamachus rufiabdominalis* emerged from the cocoons of sawflies, and the female to male ratio was 2.1: 1 (Table 2). The other ichneumonids species comprised *L. nigrus* $(8 \oplus 95 \Im \Im)$, *Goryphus* sp. $(3 \oplus 92 \Im \Im)$, *A. nigricoxa* $(2 \oplus 9)$, *Olesicampe* sp. $(1 \oplus)$, *Bathythrix* sp. $(1 \oplus)$, *Caenocryptus* sp.

Table 1. Parasitism rates (%) of parasitoids in Neodiprion huizeensis in Weining County, Guizhou Province, China.

| Collection | Cocoons - | N. huizeensis | | Ichneumonid complex | | | D. auricapita | | | Trichomalus sp. | | | D (0/) |
|------------|-----------|---------------|-----|---------------------|----|-------|---------------|----|-------|-----------------|----|-------|---------|
| | | Е | D | Е | D | P (%) | Е | D | P (%) | Е | D | P (%) | - P (%) |
| 1 | 2225 | 1063 | 372 | 340 | 36 | 16.9 | 278 | 19 | 13.3 | 88 | 29 | 5.3 | 35.5 |
| 2 | 2040 | 964 | 347 | 311 | 17 | 16.1 | 288 | 26 | 15.4 | 70 | 17 | 4.3 | 35.8 |
| 3 | 1908 | 1006 | 280 | 320 | 27 | 18.2 | 189 | 11 | 10.5 | 61 | 14 | 3.9 | 32.6 |
| Average | _ | _ | _ | _ | _ | 17.1 | _ | _ | 13.1 | _ | _ | 4.5 | 34.6 |

Notes: E: emergence; D: dissection; P: parasitism rate.

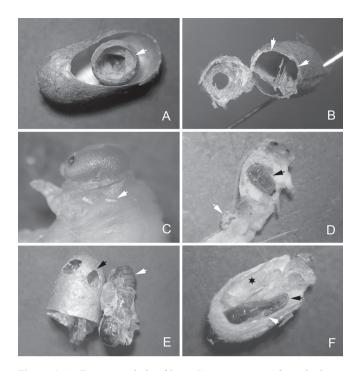


Figure 1. A. Emergence hole of larva *Drino auricapita* from the larva of *Neodiprion huizeensis* (white arrow). B. Emergence holes of larvae *D. auricapita* from the larva of sawfly (white arrow). C. Larvae of *Trichomalus* sp. parasitizing on the body of the mature instar larva of sawfly (white arrow). D. Fecal pellets of *Trichomalus* sp. (white arrow); larva of *D. auricapita* inside the body of the mature instar larva of sawfly (black arrow). E. Emergence holes of *Gelis* sp. (black arrow); larva of *D. auricapita* (white arrow). F. Silk cocoon (hexagon) and larva (black arrow) of *Gelis* sp.; Pupa of *Trichomalus* sp. (white arrow).

The main parasitoids of N. huizeensis included D. auricapita, A. melana, A. grandis, D. indica, L. rufia-

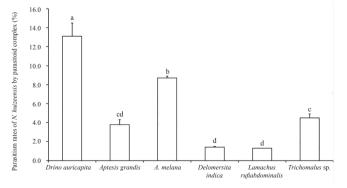


Figure 2. Mean parasitism rates (+ SE, n = 3) of *Neodiprion huizeensis* by parasitoids in Weining County, Guizhou Province. Bars with the same letter are not significantly different (ANOVA on arcsin (p)^{1/2}, followed by the Ryan-Einot-Gabriel-Welsh (REGW) multiple Q test, $\alpha = 0.05$).

bdominalis, and Trichomalus sp. The parasitism rates of N. huizeensis by these parasitoids were 13.1%, 8.7%, 3.8%, 1.4%, 1.3% and 4.5%, respectively. There were significant differences in the parasitism rates among these six species of parasitoids (F = 51.38, df = 5, 12, P < 0.001) (Fig. 2). There were significant differences of parasitism rates between D. *auricapita* and other five species (P < 0.001). There were significant differences of parasitism rates between A. melana and other 4 species (P < 0.001). There were no significant differences of parasitism rates between *Trichomalus* sp. and A. grandis (P > 0.05), but those parasitism rates of *Trichomalus* sp. being significantly higher than those of both D. indica and L. rufiabdominalis (P < 0.001), and no significant differences of parasitism rates among A. grandis, D. indica and L. *rufiabdominalis* (P > 0.05). The dominant parasitoid species of N. huizeensis were D. auricapita followed by A. melana. The adult emergence of *N*. *huizeensis* had two peaks and does not consider the male and female. The first peak occurred from 17th to 23rd February with 179 sawflies emerging on 20th February. The second peak was from 29th February to 15th March with 174 sawflies emerging on 3rd March. The number of sawflies emerging dropped gradually from 15th March and ended on 5th April 2012 (Fig. 3).

The mature larvae emergence periods of *D. auricapita* out from the sawfly cocoons were from 8th February to 24th March. There had one peak occurred from 8th to 20th February, with 140 final instar larvae emerging on 11th February. The numbers of larvae emerging dropped gradually from 14th February. The adult emergence periods of *D. auricapita* were from 20th February to 2nd April, had one peak occurred from 23rd February to 6th March, with 75 flies on 29th February. The adult emergence periods of *D. auricapita* had 43 days (Fig. 3).

The adult emergence periods of *A. grandis* were from 20th February to 9th March, with 24 adults on 29th February. The adult emergence periods of *A. melana* were from 17th February to 12th March, with 63 adults on 20th February. The adult emergence periods of *D. indica* were from 17th February to 3rd March, with 10 adults on 23rd February. The adult emergence periods of *L. rufiabdominalis* were from 3rd to 30th March, with 5 adults on 18th March (Fig. 3).

The emergence periods of ectoparasitoid *Trichomalus* sp. were from 17th February to 12th March, had one peak occurred from 23rd February to 3rd March, with 26 sawflies cocoons were parasitized by this chalcid on 26th February (Fig. 3).

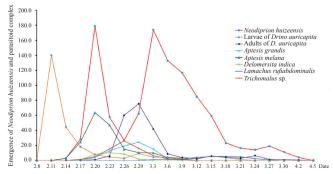


Figure 3. Adult emergence of *Neodiprion huizeensis* and its main parasitoids in 2012.

| Collections/ | A. grandis | | A. melana | | D. indica | | L. rufiabdominalis | | Others | |
|--------------|------------|-------|-----------|-------|-----------|-------|--------------------|-------|--------|----|
| Ichneumonids | F/M | SR | F/M | SR | F/M | SR | F/M | SR | F/M | SR |
| 1/340 | 48/40 | 1.2:1 | 120/71 | 1.7:1 | 20/8 | 2.5:1 | 18/9 | 2.0:1 | 6 | _ |
| 2/311 | 33/25 | 1.3:1 | 110/73 | 1.5:1 | 21/8 | 2.6:1 | 19/8 | 2.4:1 | 14 | _ |
| 3/320 | 44/41 | 1.1:1 | 105/58 | 1.8:1 | 21/9 | 2.3:1 | 16/8 | 2.0:1 | 18 | _ |
| Average | - | 1.2:1 | - | 1.7:1 | - | 2.5:1 | - | 2.1/1 | - | _ |

Table 2. Numbers and sex ratio of ichneumonid complex parasitizing on Neodiprion huizeensis, in Weining County, Guizhou Province, China.

Notes: F/M female/male; SR sex ratio.

The adults emergence of parasitoids were coincide with each other and occurring from 23rd February to 6th March, which was between the emergence peak of *N. huizeensis*.

Discussion

The average parasitism rate of *N. huizeensis* by parasitoids was 34.6%. These parasitoids play an important role to control *N. huizeensis* populations in nature. The emergence of overwintering adults of *N. huizeensis* were from middle March to late April (Xu *et al.* 1990, Ying and Luo 2002). Under the experimental conditions, these periods of *N. huizeensis* were from late February to early April.

The genus *Drino* comprises 38 described species in China (O'Hara *et al.* 2009). Most species of this genus parasitize on either sawfly or lepidopteran larvae (Shima 1999). *Drino auricapita* parasitizing on *Neodiprion sertifer* (Geoffroy, 1785), *Diprion jingyuanensis* Xiao & Zhang, 1994, in Sichuan and Shanxi Province (Chao *et al.* 1998). *Drino auricapita* was the dominant species, attacking 13.1% of the *N. huizeensis* cocoons. Tachinids are playing an important role in control sawflies.

Superparasitism in the parasitic Diptera appears to be both widely distributed among species and common within populations (Feener and Brown 1997). This phenomenon was observed frequently with *D. gilva* for the common pine sawfly (Herz and Heitland 1999). Superparasitism of the gypsy moth, *Lymantria dispar* (Linnaeus, 1758) by *Parasetigena silvestris* (Robineau-Desvoidy, 1863) to be up to 83% and that to be as high as 77% by *Blepharipa schineri* (Mesnil, 1939) (Maier 1990). The superparasitism can be advantageous when the number of unparasitized hosts is low in the field (van Alphen and Visser 1990). *Drino auricapita* is a solitary endoparasitoid of *N. huizeensis* larvae and has been observed to superparasitize *N. huizeensis* infrequently. The biology, ecology of *D. auricapita* and relationship with its host need more investigation. Ichneumonid complexes of *N. huizeensis* were including more than 12 species, of which 6 are identified as species. The unidentified species need more investigation. *Delomerista indica* is distributed in India, and no host record known (Gupta 1982). *Lamachus gilpiniae* Uchida, 1955, parasitizing on *Diprion jingyuanensis* and *Gilpinia tohi* Takeuchi, 1940, distributed in China and Japan (Uchida 1955; Li *et al.* 2012d). In our investigation, *L. rufiabdominalis* and *L. nigrus* reared from *N. huizeensis* in South China (Li *et al.* 2012a).

The parasitism rates of *N. huizeensis* by the genus *Aptesis* were about 12.5%, including 8.7% by *A. melana* which is a dominant species for *N. huizeensis* follows *Drino auricapita*. However, only two females of *A. nigricoxa* reared from the overwintering cocoons of *N. huizeensis* (Li *et al.* 2012a). *Gelis* sp. which parasitizing on *N. huizeensis* is a gregarious parasitoid, and a multiparasitoid with *Drino auricapita* or *Trichomalus* sp. One *N. huizeensis* cocoon was parasitized by an endoparasitoid *D. auricapita* and emerged two females of gregarious *Gelis* sp. Another sawfly cocoon mulitparasitized by *Gelis* sp. and *Trichomalus* sp., they all emerged from the cocoon. *Gelis* sp. parasitic characteristics and relationships with its host need more investigation.

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Table 3. Puparian periods and sex ratio of *Drino auricapita* parasitizing on *Neodiprion huizeensis*, in Weining County, Guizhou Province, China.

| Callestiana | Drino auricapita | | | | | | | | | |
|---------------|------------------|-----------|--------------------------|------------|-----------|--|--|--|--|--|
| Collections – | Pupa | Emergence | Pupal stage (Mean ± SE)* | Femal/Male | Sex ratio | | | | | |
| 1 | 278 | 243 | $16.7 \pm 0.1a$ | 125/118 | 1.1:1 | | | | | |
| 2 | 288 | 263 | $16.4 \pm 0.1a$ | 133/130 | 1.0: 1 | | | | | |
| 3 | 189 | 155 | $16.2 \pm 0.1a$ | 84/71 | 1.1:1 | | | | | |
| Average | - | _ | 16.4 ± 0.1 | - | 1.1:1 | | | | | |

Notes: * Bars with the same letter are not significantly different (ANOVA followed by the Ryan-Einot-Gabriel-Welsh (REGW) multiple Q test, at: $\alpha = 0.05$).

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