Biology and Natural Enemies of *Agrilus fleischeri* (Coleoptera: Buprestidae), a Newly Emerging Destructive Buprestid Pest in Northeast China

Kai Zang¹, Xiao-Yi Wang^{1*}, Zhong-Qi Yang¹, Ke Wei¹, Jian Jun Duan²

22

23 **Abstract:** *Agrilus fleischeri* Obenberger (Coleoptera: Buprestidae) is an emerging pest of poplar
24 trees in northeast China, and is responsible for poplar mortality throughout its range. Its biology and
25 natural enemies were studied at two field locations from 2013 to 2015 as well as via laboratory
26 experiments. Life history of *A. fleischeri* was univoltine on exotic *Populus nigra* var. *italica* and
27 overwintered as mature larvae in pupal chambers; however, was semivoltine on native *Populus*
28 *daurica* and overwintered as 2nd or 3rd instar larvae in Liaoning Province, China. Adults
29 emerged from late May to mid-August with a peak in early June. Eggs were first discovered in early
30 June and continued to late August. Larvae fed from mid-June to late September of univoltine or
31 from mid-June to the next early September of semivoltine. The sex ratio of adult beetles was
32 approximately 1:1 (M:F). Mean longevities of female and male adults were 34.87 ± 18.70 and 34.33
33 ± 18.10 days, respectively. Mean duration of egg stage was 12.83 ± 1.10 days and pupal duration
34 26.40 ± 1.32 days. Mortality in the field was mainly caused by parasitoids; however, pathogens,
35 predators, and host tree resistance were also noted. The egg parasitoid, *Oobius* spp. attacked 48.0%
36 and 6.48% of *A. fleischeri* eggs in *P. nigra* var. *italica* and *P. daurica*, respectively. *Polystenus*
37 *rugosus* and *Paramblynotus* sp. parasitized 0.71% and 52.17% of larvae in *P. nigra* var. *italica*
38 respectively. *Euderus fleischeri*, *Polystenus rugosus*, and *Spathius* sp. were found in *P. daurica*
39 with parasitism rates of 7.63%, 4.83%, and 0.25% respectively.

40 **Key words:** *Agrilus fleischeri*, life history, biology, infested traits, parasitoids

41

41

42 **Introduction**

43 China leads the world in the largest planting area of poplar trees (*Populus* spp.), encompassing
44 an area up to 69 million hm² (CSFA, 2014). However, the extensive plantings of poplar species
45 (endemic and exotic) has perturbed the forest ecosystem, resulting in increased outbreaks of various
46 plant diseases and insect pests (Weisgerber, 2002; Zhao et al., 2007). Two poplar species
47 commonly used in Chinese plantations are *Populus nigra* var. *italica* (Salicales: Salicaceae), which
48 is a fast-growing European and western Asian species (Sheng, 2014), and the native *Populus*
49 *daurica* that is widely planted in northern China. Importantly, wood products from *P. daurica*
50 are commonly used for paper-making, construction, and medical supplies (IB-CAS, 1994). Thus,
51 impacts to these important plantation species may have economic consequences of wood products
52 (Jiang et al., 2004).

53 *Agrilus fleischeri* Obenberger (Coleoptera: Buprestidae), native to East Asia, is a newly
54 emerging problematic wood-boring beetle infecting Chinese poplar plantations species (Jendek and
55 Grebennikov, 2011) as well as willow species (*Salix* spp.) in Tianjin, Beijing, and Tangshan City,
56 Hebei Province, China (XYW, personal observation). Previous investigations found that *A.*
57 *fleischeri* was the major wood-boring pest of both *P. nigra* var. *italica* and *P. daurica* in
58 plantations located in Fengcheng City, Liaoning Province, China in 2013. Larvae of this beetle
59 develop under the cambium within the phloem of infested trees. Feeding activity by larvae disrupts
60 the transportation of nutrients and water in the tree. Furthermore, when densities of the larvae are
61 high they can effectively girdle and kill the tree within 2–3 years. The host tree infestation process
62 is similar to that observed with other *Agrilus* woodborer species, such as *A. subrobustus* and *A.*
63 *anxius* (Westcott, 2007; Muilenburg and Herms, 2012). In addition, this pest can greatly reduce the

64 utilization and ornamental value of poplar trees.

65 An insect species can potentially become a severe invasive pest when it is moved to a new
66 location and separated from its natural enemy complexes, such as *Agrilus planipennis* Fairmaire
67 (Coleoptera: Buprestidae), a devastating invasive pest that has killed millions of ash trees (*Fraxinus*
68 spp.) since its accidental introduction from Northeast Asia to North America (Cappaert et al., 2005;
69 Poland and McCullough, 2006; Duan et al., 2015). *Agrilus fleischeri* has caused severe damage to
70 Chinese poplar tree plantation species, especially the non-native species, *P. nigra* var. *italica*. Thus,
71 it may have the potential to become problematic in areas where *P. nigra* var. *italica* trees are widely
72 planted.

73 Currently, the biology of *A. fleischeri* is limited to taxonomic descriptions regarding its'
74 morphological characteristics, host range, and distribution (Jendek and Grebennikov, 2011). In the
75 present study, we investigated the biology and natural enemies of this pest in northeastern China
76 from 2013-2015. Information obtained from the present study will be important for developing pest
77 management approaches, and may contribute to the identification of effective natural enemies for
78 biocontrol of this wood-boring pest.

79

80 **Materials and Methods**

81 **Study sites**

82 The study was conducted at two different sites in Saima Township, Fengcheng City, Liaoning
83 Province, China, from April 2013 to September 2015. The first site was located in Saima Village
84 (40°59' N, 124°15' E) and contained 7-year-old *P. davidiana*, while the second site contained
85 10-year-old *P. nigra* var. *italica* and was located in Nanmiao Village (40°54' N, 124°16' E). The

86 sites were located roughly 5 km apart.

87 The annual average temperature for the growing season (May to September) of the two sites
88 was approximately 21°C with a maximum of 35°C and minimum of 4°C, while the average winter
89 (December to March) temperature was around -3°C . The annual rainfall is approximately
90 800–1200 mm.

91 **Field Experiments**

92 **Sampling stands and girdling of trees.** *Populus nigra* var. *italica* trees were 14.86 ± 3.74 m
93 on average in height and the mean height of *P. davidiana* trees was about 7.19 ± 0.75 m. The
94 average diameter at breast height (DBH) of *P. nigra* var. *italica* and *P. davidiana* trees were $15.42 \pm$
95 1.64 cm and 8.83 ± 1.70 cm, respectively. Twenty *P. nigra* var. *italica* and 20 *P. davidiana* trees
96 were randomly selected and girdled on April 15th, 2013 and April 20th, 2014, respectively, for
97 attracting oviposition of *A. fleischeri*. For girdling, a 20-cm wide bark band of the tree trunk
98 (approximately 20 cm above the ground) was completely removed using a drawknife. Additionally,
99 trees that showed obvious signs and symptoms of borer-infestation were felled ($n = 5$). Overall, 25
100 *P. nigra* var. *italica* (7 were felled in 2014 and 18 in 2015) and 9 *P. davidiana* trees (felled in 2015)
101 were felled and dissected to determine the wood-boring pest larval/egg densities and associated
102 natural enemies from April 2014 to September 2015.

103 **Observations of *A. fleischeri* Life history.** The life history of *A. fleischeri* was observed from
104 April 2014 to September 2015 in the two stands. From early to late April in both years, logs were
105 dissected every three days to determine the developmental stages of immature *A. fleischeri*
106 (primarily pupae). One to three logs from the same sample tree were dissected depending on larval
107 densities in each log. Another girdled tree would be felled and dissected after finishing the

108 dissection of all logs from the previous sample tree. Dissection and peeling were stopped from late
109 May to middle June as the adult flight period. After emergence of most adults (by mid-June), we
110 continued to sample logs for determining the developmental durations of immature *A. fleischeri*.

111 **Eggs.** Field surveys were performed every two days from the middle of May to the middle of
112 June in 2014 and 2015 by observing the bark for oviposition of adult beetles.

113 **Larvae.** Observations of larval morphology and development were conducted every three days
114 during the time when larvae were active within trees in the two sample stands, *P. nigra* var. *italica*
115 and *P. davidiana*, in Saima Township, Liaoning Province, China. The bark was peeled off using a
116 screwdriver and more than 15 larvae were obtained from each field site. Overall 510 larvae were
117 collected from the regular surveys.

118 **Mortality factors.** Mortality factors were recorded when dissecting the logs from the infested
119 trees in the field. Date, host tree species, number and height above the ground of all *A. fleischeri*,
120 number of dead *A. fleischeri*, species and numbers of associated natural enemies, and other
121 mortality factors (predators, host tree resistance, and pathogen) were recorded. Host plant resistance
122 was quantified by a larval gallery showing development of callous tissues around the larvae, and
123 larvae that were surrounded by mycelium were considered as have died from pathogen infection. A
124 pupal chamber without a pupa or mature larva and having no evidence of parasitic natural enemies
125 was attributed to predation. Parasitized eggs (dark in color or *Oobius* spp. exit hole visible on the
126 surface of egg shell) on the bark were collected into a vial (1.2cm in diameter and 5.0 cm in length).
127 In this study, the percent of parasitized beetle eggs was calculated as the number of parasitized eggs
128 divided by the sum of observed eggs. Parasitized larvae were additionally collected into a vial with
129 a piece of wet filter paper to maintain moisture. Mortality due to plant resistance was calculated as

130 number of larvae killed by host defenses divided by total number of larvae in all sample trees, while
131 mortality occurring from pathogens was calculated by dividing the number of larvae infected with
132 pathogens by the total number of larvae in all sample trees, and the percentage of mortality due to
133 predation was estimated by dividing the larvae consumed by predators by total number of larvae in
134 all sample trees.

135 **Parasitism rate by natural enemies.** The parasitism rate for each parasitoid species in each
136 site was calculated as the proportion of parasitized *A. fleischeri* eggs, larvae, pupae, or adult over
137 the total number of observed individuals from each stage. In order to observe parasitism of beetle
138 eggs, poplar bark was peeled off and dissected in field from early June to early August in 2014 and
139 2015.

140 **Laboratory Experiments**

141 **Adults.** To investigate adult emergence and oviposition, five *P. nigra* var. *italica* and one *P.*
142 *dauidiana* were selected and cut into approximately 70 cm long sections, and placed into glass
143 cages (40 × 40 × 80-cm) covered with a piece of gauze cloth that was fastened with a rubber band
144 to the top of the cage to prevent escape of emerging beetles or parasitoids. The emergence of adult
145 beetles in the glass cage was observed on a daily basis, and the height above the ground for each
146 adult exit hole on the trunks was measured and recorded. Indoor temperature and humidity were
147 recorded each day with an automatic hygrothermograph (S320-TH, HUATO System Co., Ltd.,
148 Shenzhen, China) at 02:00, 08:00, 14:00, and 20:00 h. In addition, the number, height, length, and
149 width of new exit holes were recorded and marked every day to avoid duplicate records. Chamber
150 depth could be considered as a parameter for detecting the borer-infested logs because it was the
151 deepest part of the gallery. To support the quarantine inspection of poplar woods (in trade), and to

152 avoid borer-attacked wood transferred to non-native districts/countries, we measured the depths of
153 the larval overwintering chambers. Because bark was peeled from logs before measurement, the
154 depth of overwintering chamber denoted the vertical distance from the bottom of chamber to the
155 surface of sapwood. Adults emerging from the logs in the same day were collected and reared in
156 plastic cages ($17 \times 17 \times 26$ -cm). In addition, the sex of newly emerged adults was recorded every
157 day. Adults were provided with fresh poplar leaves and the leaves were replaced every other day
158 until the beetles died. The longevity of adults were calculated from emergence to death at a
159 temperature of 23.4 ± 2.3 °C, 69 ± 10 % RH. Morphological characteristics were observed and
160 recorded based on the freshly dead specimens, discrimination of males and females was on account
161 of position of mating, body length and width of dead adult beetles ($n = 220$) were measured under a
162 microscope (Motic SMZ-140, Motic China Group Co., Ltd., Xiamen, China).

163 Upon emergence, we measured mating duration in the lab using a timer (727, WUJIAO Co.,
164 Ltd., Guangzhou, China). After mating females were transferred into a new glass bottle (one pair
165 per bottle), which was covered with a layer of cheese cloth and coffee filter paper overlaid with the
166 cloth to induce the adult beetles to lay eggs on the filter paper (Duan et al, 2013; Lopez and Hoddle,
167 2013; Peterson et al., 2015). Coffee filter paper was examined daily for eggs.

168 Eggs were collected from the coffee filter paper every day during the oviposition period, and
169 placed in a vial (1.5 cm in diameter and 7.0 cm in length) that held a piece of wet filter paper (3.0
170 cm in length, 1.0 cm in width) to maintain moisture. Egg development was monitored and egg color
171 was observed using the microscope in the laboratory every day under 23.4 ± 1.8 °C, $67\% \pm 6$ % RH.
172 Egg stage duration was calculated as the time from oviposition to egg hatch.

173 Overwintering mature (full-grown) larvae were collected from the pupal chambers from late

174 April to middle May. Each larva was placed in a vial (1.5 cm in diameter and 7.0 cm in length) with
175 a piece of wet filter paper (3.0 cm in length, 1.0 cm in width), and pupal duration was calculated
176 under laboratory conditions under 17.5 ± 2.5 °C, 49 ± 11 % RH.

177 **Data analysis**

178 All data in this study were tested for normality and homogeneity prior to statistical analysis.
179 Statistical analyses were performed by SPSS 19.0 software (SPSS Inc., Chicago, USA; Zhang,
180 2011). Two sample t-tests were used to compare mean longevity, body length and width of *A.*
181 *fleischeri* adults between males and females. Chi-square test was used to examine the sex ratio of *A.*
182 *fleischeri* adults.

183

184 **Results**

185 **Field Experiments**

186 **Biology.** Significant differences were found in the life history of *A. fleischeri* on the two
187 different species of poplar. *Agrilius fleischeri* was univoltine on the the exotic poplar *P. nigra* var.
188 *italica*, and semivoltine on the native *P. davidiana* trees. Adult emergence was observed to begin in
189 late May, peaked in early June, and ended early- to mid-June in both 2014 and 2015 (Fig. 4G).
190 Females consumed poplar foliage for 6.38 days (range 2 – 11 days, n = 26) before mating, and fed
191 on the margins of poplar folia for another 6.34 days (range 2 – 12 days, n = 41) before oviposition.
192 Eggs were first observed in the field in early June, and peaked in July, later declining in mid-August
193 (2014 and 2015). Neonate larvae bored directly into the bark until reaching the cambial region
194 where they fed, often forming serpentine galleries under the bark, and with a few forming vertical
195 galleries in *P. davidiana* (Fig. 4B). The early-instar larvae were first found feeding on the cambium

196 and xylem on both species of trees in mid-June, and continued to feed until late September (2014
197 and 2015). When larval densities were high, the galleries covered both the phloem and xylem. The
198 late-instar larva excavated a pupal chamber in the xylem, and then the forepart of its body curved
199 and clung to its abdominal segments, forming a J-shaped larva (Fig. 4D). Beginning in mid-August,
200 mature J-larvae entered dormancy (possibly diapause) for overwintering and resumed development
201 to pupae in early May (Fig. 1). However, larvae in the *P. davidiana* overwintered as early instars,
202 fed during the second summer, and then emerged the following spring (Fig. 2). Full-grown *A.*
203 *fleischeri* larvae bored into the xylem and built pupal cells starting in late August and peaking in
204 late September, molted to pupae during late April to early May of the next year.

205 **Mortality factors.** Investigations in the two study sites showed that several mortality factors
206 had suppressed populations of *A. fleischeri* to varying degrees (Table 1 and Table 2). We identified
207 six native enemies (parasitoids) of *A. fleischeri* in the study sites, with three of them (*Oobius* spp.,
208 *Polystenus rugosus*, *Paramblynotus* sp.) occurring only in *P. nigra* var. *italica*, and four (*Oobius*
209 spp., *Polystenus rugosus*, *Spathius* sp., and *Euderus fleischeri*) occurring only in *P. davidiana*.

210 **Parasitoids.** *Oobius* spp. (Hymenoptera: Encyrtidae), a solitary parasitoid, parasitized 48.00%
211 of eggs in *P. nigra* var. *italica* stands and 6.48% in *P. davidiana* stands.

212 *Polystenus rugosus* Forster (Hymenoptera: Braconidae) is a solitary larval idiobiont
213 ectoparasitoid, which parasitized 0.71% and 4.83% of late-instar *A. fleischeri* larvae in *P. nigra* var.
214 *italica* and *P. davidiana* respectively.

215 *Spathius* sp. (Hymenoptera: Braconidae) is a gregarious larval idiobiont ectoparasitoid, which
216 parasitized 0.25% of larvae in *P. davidiana*.

217 *Paramblynotus* sp. (Hymenoptera: Liopteridae) is a solitary larval idiobiont ectoparasitoid,

218 which parasitized 52.17% of mature (full-grown) *A. fleischeri* larvae in *P. nigra* var. *italica*.

219 *Euderus fleischeri* Yang (Hymenoptera: Eulophidae) is a gregarious larval idiobiont
220 ectoparasitoid that parasitized 7.63% of *A. fleischeri* larvae in *P. davidiana*.

221 **Predator.** A clerid beetle (Coleoptera: Cleridae) was found to predate on 0.12% of mature
222 (full-grown) *A. fleischeri* larvae in pupal cells in *P. nigra* var. *italica* trunks.

223 **Pathogens.** The entomogenous fungi *Beauveria bassiana* (Hypocreales: Clavicipitaceae)
224 (Bals.-Criv.) Vuill. infected 6.21% and 10.43% of *A. fleischeri* larvae in *P. nigra* var. *italica* and *P.*
225 *davidiana* respectively.

226 **Host resistance.** The formation of callus tissue around *A. fleischeri* larvae lead to the death of
227 6.36% and 3.45% of larvae in *P. davidiana* and *P. nigra* var. *italica* respectively.

228 **Laboratory Experiments**

229 **Morphology.** The body of *A. fleischeri* adults is dark brown to black, glabrous, and the two
230 white elytral spots are elongate oblong and lack tomentose, adsutural spots at 1/3 portion apically of
231 elytra is "V"-shaped arranged. The prehumeral is short, with anterior apex distant from lateral
232 pronotal margin. The face of the male is green, and that of the female is brown (Fig. 5B). The
233 pectinate antennae have 11 segments, and the kidney-shaped ommatidium is black. The body size of
234 males was significantly smaller than that of females ($t = 5.318$, $df = 218$, $P < 0.001$), with the
235 average length of males being 10.12 ± 0.83 mm (range 7.87 – 11.48 mm, $n = 104$), and that of
236 females 10.80 ± 1.02 mm (range 7.80 – 12.33 mm, $n = 116$). The average width of males was
237 significantly less than females ($t = 9.047$, $df = 218$, $P < 0.001$) and was measured at 2.62 ± 0.23 mm
238 (range 1.84 – 3.05 mm, $n = 104$), while females were 2.96 ± 0.31 mm (range 1.84 – 3.40 mm, $n =$
239 116) in width (Fig. 5A).

240 **Adults.** Emerged adults left a distinct D-shaped exit hole in the trunk, which was on average
241 2.97 ± 0.37 mm in length (range 2 – 4 mm, $n = 190$), and 2.01 ± 0.23 mm in width (range 1.3 – 2.8
242 mm, $n = 190$)(Fig. 4H). In the early stages of eclosion, numbers of emerged males exceeded
243 females, whereas at peak and later, the number of females was greater than the number of males
244 (Fig. 3). A sex of 0.897:1 (M:F) was calculated from the emerged adults and was not significantly
245 different from a theoretical sex ratio of 1:1 ($\chi^2 = 0.655 < \chi^2_{0.05, 1} = 3.84$, $P = 0.418$). The average
246 longevity of males was 34.33 ± 18.10 days (range 2 – 82 days, $n = 93$) and females was $34.87 \pm$
247 18.70 days (range 2 – 73 days, $n = 100$); however, there was no significant difference between their
248 mean longevity ($t = 0.201$, $df = 1, 191$, $P = 0.841$). Mating duration averaged 11.74 ± 1.02 minutes
249 ($n = 26$). Females ($n = 53$) produced average of 218 eggs (range 0 – 614 eggs) through their lifespan
250 and laid an average of 3.93 ± 1.82 eggs per day. Eggs were laid either together or individually
251 within bark cracks and crevices or beneath bark flakes (Fig. 4A).

252 **Eggs.** Newly deposited eggs were milky white, then turned to faint yellow, and finally changed
253 to brown before hatching. The eggs were oval and irregular in shape, and were on average $1.12 \pm$
254 0.29 mm in length (range 0.93 – 1.33 mm, $n = 140$) and 0.72 ± 0.19 mm in width (range 0.61 – 0.88
255 mm, $n = 140$). The eggs took 12.83 ± 1.10 days (range 10 – 15 days, $n = 119$) to hatch.

256 **Larvae.** *Agrilus fleischeri* larvae were milky white to light yellow in color, but the mouthparts
257 and urogomphi were dark brown (Fig. 4C). The ten abdominal segments were pale cream colored
258 and trapezoidal in form, making the pupa overall bell-shaped. The pronotal groove bifurcated
259 posteriorly and the prosternal groove was entire.

260 **Pupae.** Pupae were exarate, and their initial color was milky white, with the eyes first changing
261 to black, and then the elytra color also changing to black (Figs. 4E and 4F). The average pupal

length was 11.18 ± 1.05 mm (range 8.93 – 13.32 mm, n = 31) and the average width was 3.12 ± 0.29 mm (range 2.48 – 3.83 mm, n = 31). *Agrilus fleischeri* overwintered as J-shaped larvae in pupal chambers that were 6.88 ± 0.10 mm (range 4 – 14 mm, n = 207) on average from the surface of the sapwood. Eclosion from pupa to adult took 26.40 ± 1.32 days (range 25 – 29 days, n = 12).

Discussion

Agrilus fleischeri had a univoltine lifecycle on its native host *P. nigra* var. *italica*, but a semivoltine on its exotic host *P. davidiana* trees. We hypothesize that different levels of host tree resistance between the native and exotic poplars may be responsible for the difference in the observed life history of the pest. Higher levels of tree resistance in its native host may have slowed the larval development so that a second year of development is required to reach the mature stage for obligatory diapauses for overwintering. A semivoltine life history with other *Agrilus* species such as EAB had been documented in association with healthy trees at low pest densities (Tluczek et al., 2011). Alternatively, difference in nutrient levels between the two different poplar trees may also result in differential development rate of both larvae and adults and thus lead to different life cycle in *A. fleischeri*. Further studies are needed to evaluate the impact of host tree resistance or nutritional components on the life history strategy, which may have important implications for our understanding of population dynamics of this pest.

Biological control is an effective approach to managing pest populations, especially for the concealed wood-boring pests (Yang, 2004; Gould et al., 2011; Duan et al., 2012). It is helpful to improve the efficiency of biological control by making full use of natural enemies (Wang and Yang, 2008). For example, *Rhizophagus grandis* Gyllenhal successfully suppressed populations of

284 *Dendroctonus valens* LeConte which had killed millions of pine trees in China (Zhao et al., 2008).
 285 Larvae of the invasive *Eucalyptus* longhorned beetle *Phoracantha semipunctata* (Fabricius) could
 286 be readily parasitized by *Syngaster lepidus* Brulle', *Jarra phoracantha* Austin, Quicke, and Marsh,
 287 *Jarra painei* Austin and Dangerfield (Paine et al., 2000). In this study, we discovered five
 288 parasitoids (*Oobius* spp., *Spathius* sp., *Polystenus rugosus*, and *Euderus fleischeri*) associated with
 289 *A. fleischeri* infesting *P. davidiana* trees, and three other species (*Oobius* spp., *P. rugosus*, and
 290 *Paramblynotus* sp.) from the infested *P. nigra* var. *italica* trees. The dominant parasitoids were
 291 *Oobius* spp. and *Paramblynotus* sp. in the *P. nigra* var. *italica* stand, while those were *Oobius* spp.
 292 and *Euderus fleischeri* in the *P. davidiana* stand, which would play an important role in the
 293 biological control of *A. fleischeri*. In addition, high parasitism rates of these parasitoids suggested
 294 that they had the potential to manage this buprestid beetle if it does invade new areas.

295 Egg parasitoids have been generally considered as effective natural enemies to protect host trees
 296 against wood-boring pests because they have the potential to neutralize the pest before feeding
 297 damage is done to the host tree. For example, *Avetianella longoi* Siscaro (Hymenoptera:
 298 Encyrtidae), an egg parasitoid of *Phoracantha semipunctata* Describer (Coleoptera: Cerambycidae),
 299 has been successfully introduced to the US for biological control (Luhring et al., 2000). Our study
 300 showed that *Oobius* spp., an egg solitary parasitoid, attacked nearly 50% of *A. fleischeri* eggs in the
 301 field and may have the potential for successful suppression of this buprestid. However, the biology
 302 and methods for this particular egg parasitoid need to be further developed. After examination
 303 under a microscope, we confirmed that *Oobius* spp. discovered in *P. nigra* var. *italica* differed from
 304 those in *P. davidiana*. Moreover, further research will be conducted to ensure the specific species
 305 and host specialization of the two *Oobius* spp.

306 *Paramblynotus* sp., a solitary larval parasitoid through investigation, was only found in the *P.*
307 *nigra* var. *italica* stand. It caused high rates of *A. fleischeri* larval parasitism and the female
308 parasitoid preferred to parasitized the older larvae or prepupae in the pupal chambers, leaving a
309 circular exit hole in the bark. In addition, *Paramblynotus fraxini* Yang et Gu was found to
310 parasitized the larva of one or both wood borers *Mesosa myops* (Dalman) and *Tremex simulacrum*
311 Sem. on the trunk of an ash tree, *Fraxinus mandshurica* Rupr. (Yang and Gu, 1994). The long
312 ovipositors of females in family Liopteridae indicated that they have more potential in successfully
313 suppressing populations of wood borers for biological control.

314 *Agrilus fleischeri* is responsible for extensive damage to the economically important poplar tree
315 in northeast China. Given the limitation of other control methods, the high parasitism rates of *A.*
316 *fleischeri* by the observed parasitoids in our study suggest that biological control could be a
317 potentially effective approach to managing this pest species in northeast China.

318

319 **Acknowledgments**

320 This research was funded by UDSA-ARS Agreement (58-8010-5-001F) with the Chinese
321 Academy of Forestry (Xiao-Yi Wang). We thank Yun-Cheng Liu and Zhi-Gang Liu for assistance
322 in field girdling the sample trees. We appreciate comments on an early draft of this manuscript by
323 Julian Golec and Shang-Kun Gao. We thank two anonymous reviewers for their valuable
324 suggestions.

325

326 **References**

327 (CSFA) China State Forestry Administration, 2014. The Forest resources inventory results by the

328 Eighth National Survey (2009-2013). China Forestry Website <http://www.forestry.gov.cn/>.

329 Duan, J.J., Bauer, L.S., Abell, K.J., Van Driesche, R.G., 2012. Population responses of
 330 hymenopteran parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded
 331 areas in north central United States. *Biol. Control.* 57, 199-209.

332 Duan, J.J., Watt, T., Taylor, P., Larson, K., Lelito, J.P., 2013. Effects of ambient temperature on
 333 egg and larval development of the invasive emerald ash borer (Coleoptera: Buprestidae):
 334 implications for laboratory rearing. *J. Econ. Entomol.* 106, 2101-2108.

335 Duan, J.J., Bauer, L.S., Abell, K.J., Ulyshen, M.D., Van Driesche, R.G., 2015. Population dynamics
 336 of an invasive forest insect and associated natural enemies in the aftermath of invasion:
 337 implications for biological control. *J. Appl. Entomol.* 52, 1246-1254.

338 Gould, J.R., Ayer, T., Fraser, I., 2011. Effects of rearing conditions on reproduction of *Spathius*
 339 *agrili* (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer (Coleoptera:
 340 Buprestidae). *J. Econ. Entomol.* 104, 379-387.

341 (IB-CAS) Institute of Botany, Chinese Academy of Sciences, 1994. Illustration of Senior Plants in
 342 China. Science Press, Beijing.

343 Jendek, E., Grebennikov, V.V., 2011. *Agrilus* of East Asia. Jan Farkač, Prague.

344 Jiang, Y.Z., Wang, G.Y., Lv, L.C., Yuan, S.P., Ma, L., 2004. Studies on pulporiented cultivation
 345 techniques of poplar wood. *Sci. Silvae Sin.* 40, 123-130.

346 Lopez, V.M., Hoddle, M.S., 2013. Mortality factors affecting *Agrilus auroguttatus*, Schaeffer
 347 (Coleoptera: Buprestidae) eggs in the native and invaded ranges. *Biol. Control.* 67, 143-148.

348 Luhring, K.A., Paine, T.D., Millar, J.G., Hanks, L.M., 2000. Suitability of the eggs of two species
 349 of Eucalyptus Longhorned Borers (*Phoracantha recurva*, and *P. semipunctata*) as hosts for the

350 encyrtid parasitoid *Avetianella longoi*. Biol. Control. 19, 95-104.

351 Muilenburg, V.L., Herms, D.A., 2012. A review of bronze birch borer (Coleoptera: Buprestidae)

352 life history, ecology, and management. Environ. Entomol. 41, 1372-1385.

353 Paine, T.D., Paine, E.O., Hanks, L.M., Millar, J.G., 2000. Resource partitioning among parasitoids

354 (Hymenoptera: Braconidae) of *Phoracantha semipunctata*, in their native range. Biol. Control. 19,

355 223-231.

356 Peterson, D.L., Duan, J.J., Yaninek, J.S., Ginzel, M.D., Sadof, C.S., 2015. Growth of larval *Agrilus*

357 *planipennis* (Coleoptera: Buprestidae) and fitness of *Tetrastichus planipennisi* (Hymenoptera:

358 Eulophidae) in blue ash (*Fraxinus quadrangulata*) and green ash (*F. pennsylvanica*). Environ.

359 Entomol. 44, 1512-1521.

360 Poland, T.M., McCullough, D.G., 2006. Emerald ash borer: invasion of the urban forest and the

361 threat to North America's ash resource. J. For. 104, 118-124.

362 Sheng, W.T., 2014. Plantation Forest and Their Silviculture Systems in China. China Forestry

363 Publishing House, Beijing.

364 Tluczek, A.R., McCullough D.G., Poland T.M., 2011. Influence of host stress on emerald ash borer

365 (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) adult density, development, and

366 distribution in *Fraxinus pennsylvanica* trees. Environ. Entomol. 40, 357-366.

367 Wang, X.Y., Yang, Z.Q., 2008. Behavioral mechanisms of parasitic wasps for searching concealed

368 insect hosts. Acta Ecol. Sin. 28, 1257-1269.

369 Weisgerber, H., 2002. Establishing poplar plantation and its risk. World For. Res. 15, 14-19.

370 Westcott, R.L., 2007. The exotic *Agrilus subrobustus* (Coleoptera: Buprestidae) is found in northern

371 Georgia. Coleopt. Bull. 61, 111-112.

372 Yang, Z.Q., 2004. Advance in bio-control researches of the important forest insect pests with
373 natural enemies in China. Chin. J. Biol. Control. 20, 221-227.

374 Yang, Z.Q., Gu, Y. Q., 1994. Liopteridae (Hymenoptera: Cynipoidea) from China with description
375 of a new species. Entomotaxonomia. 16, 157-164.

376 Zhang W.T., 2011. SPSS statistical analysis based tutorial. Higher Education Press, Beijing.

377 Zhao, J.X., Yang, Z.Q., Liang, T.J., 2008. Research advance on biological control of conifer bark
378 beetles with the predator, *Rhizophagus grandis* (Coleoptera: Rhizophagidae). Sci. Silvae Sin. 44,
379 151-156.

380 Zhao, T.H., Zhao, W.X., Gao, R.T., Zhang, Q.W., Li, G.H., Liu, X.X., 2007. Induced outbreaks of
381 indigenous insect species by exotic tree species. Acta Entomol. Sin. 50, 826-833.

382

382 **Table 1.** Mortality factors for natural populations of *Agrilus fleischeri* on *Populus nigra* var. *italica*

Stage	Total number (n)	Mortality factors	Mortality (%)
Egg	1,973	<i>Oobius</i> spp.	48.00
		Predator	1.82
		Unhatched	5.58
		Unknown factors	1.47
Larva	1,014	<i>Polystenus rugosus</i>	0.71
		<i>Paramblynotus</i> sp.	52.17
		Predator	0.12
		<i>Beauveria bassiana</i>	6.21
		Resistance	3.45
		Unknown factors	6.11
Adult	462	Unknown factors	6.71

383

383

384 **Table 2.** Mortality factors for natural populations of *Agrius fleischeri* on *Populus davidiana*

Stage	Total number (n)	Mortality factors	Mortality (%)
Egg	509	<i>Oobius</i> spp.	6.48
		Unhatched	1.77
		Unknown factors	0.98
Larva	393	<i>Polystenus rugosus</i>	4.83
		<i>Spathius</i> sp.	0.25
		<i>Euderus fleischeri</i> n. sp.	7.63
		Cannibalism	2.54
		<i>Beauveria bassiana</i>	10.43
		Resistance	6.36
		Unknown factors	5.34
Adult	36	Unknown factors	11.11

385

385

386 **Fig. 1.** Life history of *Agrilus fleischeri* on *Populus nigra* var. *italica*. (Fengcheng, Liaoning

387 Province, 2014-2015)

388 Figure legends: E, M, and L denote the early, middle, and late period (ten days) of a month. ○, pupa.

389 +, adult. ·, egg. Δ, larva. ▲, overwintering larva.

390

391 **Fig. 2.** Life history of *Agrilus fleischeri* on *Populus davidiana*. (Fengcheng, Liaoning Province,

392 2014-2015)

393 Figure legends: E, M, and L denote the early, middle, and late period (ten days) of a month. ○, pupa.

394 +, adult. ·, egg. Δ, larva. ▲, overwintering larva.

395

396 **Fig. 3.** The relationship between dates and numbers of emerging *Agrilus fleischeri* adults.

397 Figure legends: female, imaginary line; male, full line.

398

399 **Fig. 4.** *Agrilus fleischeri* life stages and behavior. (A) Eggs. (B) Larva in early instar. (C) Larva in

400 late instar. (D) J-shaped larva. (E) Prepupa. (F) Pupa. (G) Newly emerged Adult. (H) D-shaped exit

401 hole.

402

403 **Fig. 5.** *Agrilus fleischeri* adult. (A) Size difference between sexes. (B) Color difference between

404 two sexes in face.

405

406

407

Date \ Stage	Jan.-Apr.			May			Jun.			Jul.			Aug.			Sep.			Oct.-Dec.		
	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L
Overwintering larva	▲	▲	▲	▲																	
Pupa				⊖	⊖	⊖	⊖														
Adult							+	+	+	+	+	+	+	+	+						
Egg							.	.	.	.	.	.	.	.	.						
Larva							Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ			
Overwintering larva																▲	▲	▲	▲	▲	▲

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

A

B

♀

♂

462

Fig. 5.

Eggs

Larva

J-shaped
larva

Pupa

Adult