Natural enemies of emerald ash borer (Coleoptera: Buprestidae) in northeast China, with notes on two species of parasitic Coleoptera

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Abstract—To investigate natural enemies of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in northeastern China, we conducted field surveys of ash (*Fraxinus* Linnaeus (Oleaceae)) trees in semi-natural forests and plantations at variable EAB densities from 2008 to 2013. Our surveys revealed a complex of natural enemies including eight hymenopteran parasitoids and two apparently parasitic Coleoptera, woodpeckers, and several undetermined mortality factors. Parasitoid complex abundance and its contribution to EAB mortality varied with the time of year, type of ash stands, and geographic regions. The egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) and the larval parasitoid *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) were frequently observed in Jilin, Liaoning, and Heilongjiang provinces and in Beijing, but not in Tianjin. *Spathius agrili* Yang (Hymenoptera: Braconidae), however, was more prevalent near Beijing and further south in Tianjin. Larvae of two species of apparently parasitic beetle, *Tenerus* Laporte (Coleoptera: Cleridae) species and *Xenoglena quadrisignata* Mannerheim (Coleoptera: Trogossitidae), were also recovered attacking overwintering EAB in Liaoning Province, with *Tenerus* species being a dominant mortality agent (~13%). Our findings support the need to consider the geographic origin of insect natural enemies for EAB biocontrol, as well as an expanded foreign exploration for EAB natural enemies throughout its native range in Asia.

Introduction

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive insect pest of ash (*Fraxinus* Linnaeus; Oleaceae) trees, introduced to North America from Asia (Haack *et al.* 2002). Since initial detection in Michigan, United States of America in 2002, this pest has been found in 24 other states and two Canadian provinces, killing tens of millions of ash trees and causing severe damage to forested ecosystems (Cappaert *et al.* 2005; Poland and McCullough 2006; Herms and McCullough 2014; Bauer *et al.* 2015). Most recently, Orlova-Bienkowskaja (2014) proposed that EAB infestations detected in the European region of Russia would likely expand throughout Europe. Early EAB eradication efforts in North America were unsuccessful, and chemical control is expensive and environmentally unacceptable in natural forests. Therefore, efforts to manage EAB now emphasise the use of classical biological control

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(Bauer *et al.* 2008, 2015; Duan *et al.* 2010, 2013; Abell *et al.* 2014). This well accepted, long-term, and sustainable approach to the management of invasive species, involves the introduction and establishment of co-evolved natural enemies from the native range of an invasive pest (Van Driesche *et al.* 2008; Van Driesche 2014).

Following the invasion of North America by EAB, field surveys of EAB natural enemies were conducted in low to heavily infested ash stands in plantations, city parks, and in semi-natural rural and urban forests in the native range of EAB in northeast China, Russian Far East, and South Korea (Liu *et al.* 2003, 2007; Duan *et al.* 2012c; Bauer *et al.* 2014, 2015). These field surveys led to the discovery and description of several species of hymenopteran parasitoids: the egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang *et al.* 2005), and the larval parasitoids: *Spathius agrili* Yang (Hymenoptera: Braconidae) (Liu *et al.* 2003; Yang *et al.* 2005), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Yang *et al.* 2006), *Sclerodermus pupariae* Yang and Yao (Hymenoptera: Bethylidae) (Yang *et al.* 2012) from China, and *Spathius galinae* Belokobylskij and Strazenac (Hymenoptera: Braconidae) from Russian Far East and South Korea (Belokobylskij *et al.* 2012; Duan *et al.* 2012c). Other hymenopteran parasitoids of *Agrilus* Curtis species known to attack EAB in Asia and Europe are summarised in Bauer *et al.* (2015).

In an effort to manage EAB in the United States of America, introductions of three parasitoid species from China, *O. agrili*, *S. agrili*, and *T. planipennisi*, began in Michigan in 2007 and subsequently expanded to other states (Federal Register 2007; Bauer *et al.* 2008, 2014, 2015). By 2009, the introduced parasitoids were recovered at many of these early release sites, prompting the United States Department of Agriculture to initiate an EAB biocontrol programme and construct a parasitoid-rearing facility, leading to the expansion of EAB biocontrol to other EAB-infested regions of North America (Bauer *et al.* 2015).

Researchers evaluating EAB population dynamics at long-term study sites in Michigan have discovered that only two species, *O. agrili* and *T. planipennisi,* have successfully established stable and increasing populations (Duan *et al.* 2013, 2014; Abell *et al.* 2014). Besides the introduced parasitoids attacking EAB in North America, the results of recent field studies have also revealed that some native parasitoids of wood-boring larvae, such as species of *Atanycolus* Förster (Hymenoptera: Braconidae) and *Spathius* Nees (Hymenoptera: Braconidae), have acquired EAB as a novel host (Cappaert and McCullough 2009, 2010; Duan *et al.* 2009, 2010, 2011, 2012a; Marsh *et al.* 2009; Taylor *et al.* 2012; Bauer *et al.* 2014, 2015). However, these parasitoids are apparently opportunists, more specific to a niche than to host. Moreover, they are associated with high density EAB populations in some heavily infested areas and may not significantly suppress EAB populations at the leading edge of the invasion or in the aftermath forest where EAB densities are still low (Bauer *et al.* 2014, 2015; Duan *et al.* 2014). Woodpecker predation was also higher at sites with more dense EAB populations (Jennings *et al.* 2013). Thus, co-evolved natural enemies associated with low pest densities in the native range of EAB may be more effective at suppressing the target population than generalist and opportunistic natural enemies that capitalise on pests during outbreaks.

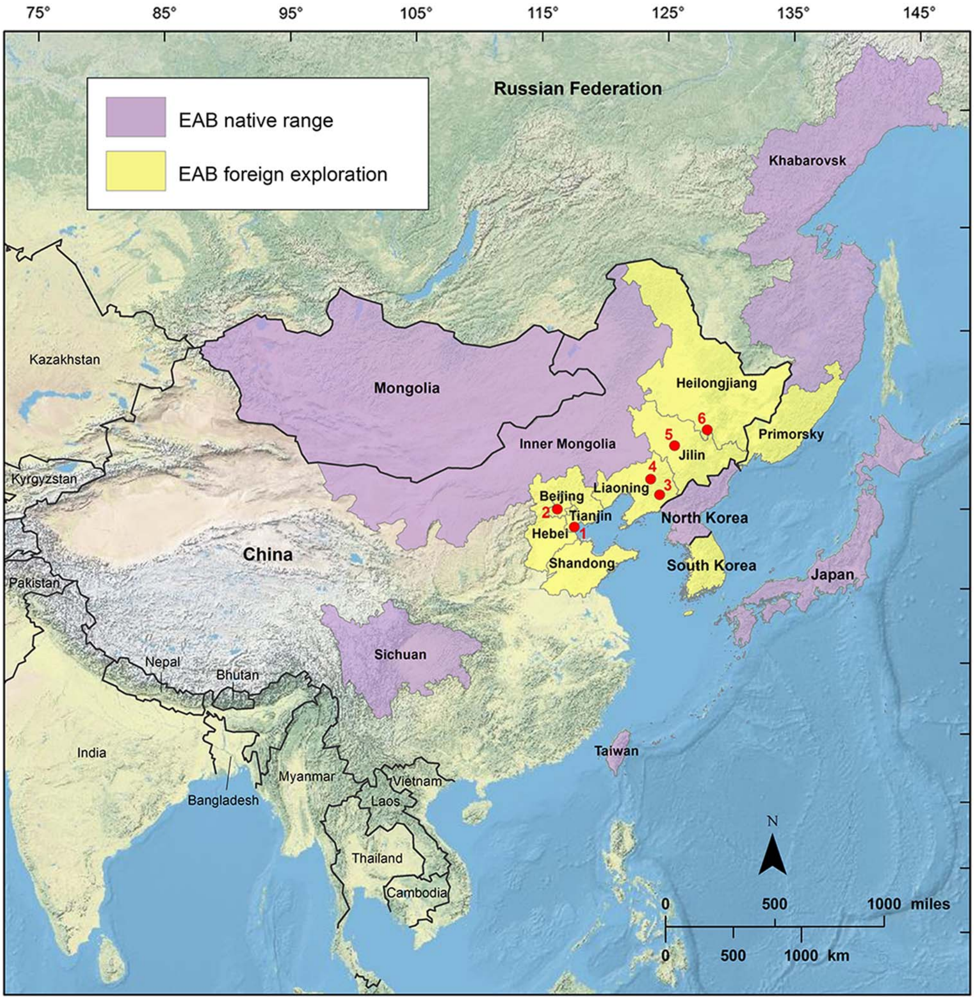
Here we report on EAB natural enemy surveys conducted from 2008 to 2013 in EAB-infested ash stands in northeastern China where EAB densities were generally low in native ash trees (mainly *Fraxinus mandshurica* Ruprecht) or high in exotic ash trees, mainly *F. velutina* Torrey. Based on the results of our surveys, we assessed the relative abundance and rate of EAB stage-specific mortalities caused by different groups of natural enemies in relation to different host ash tree species, geographic regions, and seasonality in China.

Materials and methods

Study sites

Field surveys were conducted in north and northeast China at six study sites in the two provincial cities of Beijing and Tianjin and in the provinces of Liaoning, Jilin, and Heilongjiang from 2008 to 2013 (Fig. 1, Table 1). Native *F. mandshurica* and exotic *F. velutina* trees growing in parks, semi-natural forests or monoculture plantations were sampled for EAB eggs and larvae. Because the EAB can develop in one or two years

Fig. 1. Sample locations in China. The native range of EAB in Asia as reported in the literature, and regions of Asia where EAB natural enemies were surveyed prior to this report. The red dots indicate EAB study sites surveyed for natural enemies from 2008 to 2013, with the numbers corresponding to the following locations: 1. Dagang, Tianjin; 2. Haidian, Beijing; 3. Fengcheng, Liaoning; 4. Shenyang, Liaoning; 5. Changchun, Jilin; 6. Shangzhi, Heilongjiang. Numbers on the top line of graph frame are east longitude degrees, while numbers on the right side are north latitude degrees. Service layer credits: United States National Park Service. Data source for the native range of EAB: https://sites.google.com/site/eduardjendek/world-distribution-of-agrilus-planipennis. Map created by United States Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry, Office of Knowledge Management (Durham, New Hampshire, United States of America).



depending on climate, surveys of EAB larvae and associated mortality factors were conducted in either spring or autumn, or both seasons every year. Emerald ash borer eggs and associated natural enemies were also sampled in summer seasons. The life cycle of EAB varies with different study regions. For example, EAB is univoltine in Beijing and Tianjin, but a mixture of both univoltinism and semivoltinism occurs in Liaoning Province. In contrast, a majority of EAB populations exhibit semi-voltinism in Jilin and Heilongjiang provinces (Wei *et al.* 2007; Wang *et al.* 2010).

The ash species, sizes, and age, forest type as well as level of EAB infestations varied with study sites across different provinces or cities.

Table 1. Survey sites and forest habitat description.

Sampling site	Survey date	Latitude (N)	Longitude (E)	Altitude (m)	Forest type	Ash species	Tree age (year)	Number of trees sampled	Survey target	Diameter at breast height (cm)	Notes
Dagang, Tianjin	2–4 July 2010	38°55.547′–38°56.175′	117°31.007′–117°32.103′	3.8–4.5	Plantation	*F. velutina*	13–17	10	Eggs	2.7–10.0	Naturally infested trees
	27 April to 2 May 2011							10	Larvae		
	23 October to 6 November 2011							8	Eggs, larvae		
	28 March to 1 April 2012							2, 5	Eggs, larvae		
Haidian, Beijing	27 October 2010	40°00.323′–40°00.723′	116°10.149′–116°11.182′	304.0–447.0	Semi-natural forest	*F. velutina*	6–16	8	Eggs, larvae	3.5–12.0	Naturally infested trees
	27 May 2011							5	Eggs, larvae		
	2 August 2012							12	Eggs		
Fengcheng, Liaoning	26–27 September 2008	40°52.823′–40°53.445′	124°16.419′–124°17.750′	370.4–489.2	Semi-natural forest	*F. mandschurica*	12–21	9	Larvae	4.4–17.7	Girdled trees
	24 September 2010							21, 12	Eggs, larvae		
	15 July 2011							13	Eggs		
	15–16 May 2012							5	Eggs, larvae		
	16 May 2013							6	Larvae		
Shenyang, Liaoning	14 May 2013	41°48.910′	123°33.809′	61.1	Plantation	*F. velutina*	8–10	7	Larvae	4.5–7.2	Naturally infested trees
Changchun, Jilin	20–22 Septemebr 2008	43°46.232′	125°27.348′	486.9	Plantation	*F. mandschurica*	10–13	6	Larvae	4–10	Naturally infested trees
Shangzhi, Heilongjiang	18–19 June 2012	44°40.266′	128°03.375′	283.6	Plantation	*F. mandschurica*	> 25	12, 7	Eggs, larvae	8–35	Naturally infested trees

In general, levels of EAB infestation on both *F. velutina* and *F. mandschurica* trees in either plantation or semi-natural forests were relatively low in Beijing, Liaoning, Jilin, and Heilongjiang provinces and no chemical control measures were undertaken against EAB there during the course of these surveys. At one of the study sites (Saima Township, Fengcheng) in Liaoning Province, EAB infestations on *F. mandschurica* trees in a semi-natural, managed forest, were rarely found. At this site, we girdled 10–15 *F. mandshurica* trees approximately 1.5 years prior to sampling EAB and its associated parasitoids every year during 2007–2012. In Tianjin, populations of EAB frequently reached outbreak levels in *F. velutina* plantations, where chemical control measures were undertaken once or twice a year against EAB, although trees in our sample stands were not treated.

Sampling: natural enemies associated with EAB eggs

For sampling eggs, sections of bark from two to 21 trees for each site, season, and year combination was removed from the main trunk within 2 m above the ground using a drawknife and returned to the laboratory for examination. In addition, the trunk of stressed ash trees within a 2-m section above the ground was visually searched for approximately one hour per site by three observers. Eggs were counted and examined either in the field with assistance of a hand-held magnifying lens or in the laboratory under a dissecting microscope. The fate of each EAB egg was classified according to methods described in Liu *et al.* (2007) and Duan *et al.* (2012b): (1) hatched EAB larva (egg shell brown, full of larval frass with exit hole on bottom); (2) parasitised by *O. agrili* (egg shell usually black, with small round emergence hole on top if parasitoid already emerged); (3) preyed upon by unknown predators (remaining egg shell with jagged feeding damage; and (4) died from unknown factors or infertile (unhatched dead eggs dry and flattened without frass inside the egg shell).

Natural enemies associated with EAB larvae/prepupae

At each site, 5–12 trees were felled every sampling period and the main trunk of each tree was cut into 1-m-long sections. The logs were then returned to laboratory and debarked with a drawknife. Upon debarking, the number of immature EAB stages and their fates were determined using methods described by Liu *et al.* (2003, 2007) and Duan *et al.* (2010). Parasitism of immature EAB stages by different species or groups of parasitoids was based on the association of parasitoid egg, larvae, cocoons and/or adults with dead EAB remains in the EAB gallery or pupation chamber. The parasitoid larvae, pupae or cocoons were collected and reared to adult stage in laboratory for further identification. In addition to parasitism, we recorded two other categories of mortality associated with EAB larvae and pupae: (1) woodpecker predation and (2) undetermined mortality. Woodpecker predation was determined based on visual evidence of missing or partially consumed EAB associated with destruction of bark and sapwood caused by woodpecker feeding (Lindell *et al.* 2008; Duan *et al.* 2012c, 2014). Dead EAB larvae associated with fungi, bacteria, or covered with tree callus tissues were assigned to the "undetermined biotic factors" (Duan *et al.* 2012c, 2014).

Insect identification

The identification of *Tenerus* Laporte (Coleoptera: Cleridae) species was determined by Dr. Gan-Yan Yang at the Institute of Zoology, Chinese Academy of Science, Beijing, China; and *Xenoglena quadrisignata* Mannerheim (Coleoptera: Trogossitidae) was identified by author L.M.C. The hymenopteran parasitoids were identified by author Z.Q.Y. All voucher specimens were deposited in the Insect Museum, Chinese Academy of Forestry, Beijing, China.

Statistical analyses

Data analyses were performed with SAS software Version 10.0.0 (SAS Institute Inc. 2012). Likelihood χ^2 tests were used to compare the percentages of EAB eggs parasitised by *O. agrili*, and the other mortality factors among sample sites and sampling times, as well as total EAB larval mortality at different sites (SAS Institute Inc. 2012). To compare the effects of parasitoids, woodpeckers, and other undetermined biotic factors, we calculated the total real parasitism rates of EAB larvae by all parasitoids combined for each site separately. Real parasitism is calculated as the ratio of the parasitised host number relative to the total number of the host larvae observed. To evaluate the contribution of each parasitoid species to host mortality, we calculated the larval parasitism rate of each species using the apparent parasitism for each site. Apparent parasitism is calculated by excluding from the data analysis those EAB larvae preyed on by woodpeckers (unable to determine if these larvae were parasitised before predation) and individuals that died as first instars or second instars (parasitoids generally parasitise third instars and fourth instars).

Results

Natural enemies of EAB eggs

The egg parasitoid *O. agrili* was consistently recovered from EAB eggs collected from both *F. mandschurica* and *F. velutina* grown in both semi-natural forests and ash plantations at all sample sites except Tianjin. Parasitism of EAB eggs by *O. agrili* varied significantly with sampling times (*e.g.*, from 4.0% in winter 2011 to 25.0% in summer 2012 in Beijing) as well as sample locations (*e.g.*, 0% in Tianjin and 32% in Liaoning) (Table 2). Among all the study sites, parasitism of EAB eggs by *O. agrili* was highest in Fengcheng, Liaoning (4.6–32% during 2010–2012). In addition to parasitism by *O. agrili*, predation and/or undetermined factors also caused varying levels of EAB egg mortality across different study sites and sampling times – *e.g.*, 16.8–21.9% in Tianjin from 2010 through 2012, 18.8–49.0% in Beijing (2010–2012), 22.7–46.6% in Liaoning (2010–2012), and 19.1% in Heilongjiang in 2012 (Table 2). The levels of parasitism and other mortality factors on EAB eggs also differed among years in both Beijing and Liaoning sites (df = 1, $\chi^2 = 8.1535$, $P = 0.0043$ for egg parasitism in Beijing; df = 1, $\chi^2 = 6.1446$, $P = 0.0132$ for other mortality in Beijing; df = 2, $\chi^2 = 17.4467$, $P = 0.0002$ for egg parasitism in Liaoning; df = 2, $\chi^2 = 12.4220$, $P = 0.0020$ for other mortality in Liaoning) (Table 2).

Natural enemies of EAB larvae

A total of 2682 EAB larvae from 88 girdled or naturally infested ash trees, were sampled in Tianjin, Beijing, Liaoning, Jilin, and Heilongjiang from 2008 to 2013 (Table 3). Among the EAB larvae observed at different sampling times and sites, a range of 3.0–51.6% were parasitised by different groups of larval parasitoids, 0–33.1% were preyed on by woodpeckers, and 7.8–62.0% killed by other undetermined biotic factors (*e.g.*, diseases, competition, and putative plant resistance (*i.e.*, EAB larvae killed by growth of host plant tissue or phytochemicals)). There were significant variations in larval parasitism and woodpecker predation rates as well as other undetermined biotic and abiotic mortality factors.

Hymenoptera. A total of six species of parasitic wasps (Hymenoptera) and two species of beetles with apparently parasitic larvae (Coleoptera) were recovered from or in association with EAB larvae (mostly 3rd–4th instars) and/or prepupae at different study sites (Table 4). Parasitism and distribution of the different species of parasitoids varied greatly between sampling locations but less so among years at the same location. For example, the braconid wasp *S. agrili* was the most prevalent parasitoid attacking EAB larvae in both Beijing in 2010–2011 (44.1% apparent parasitism) and Tianjin in 2011–2012 (36.8–66.7% apparent parasitism), whereas few EAB larvae were parasitised by *S. agrili* in the northeast provinces. In contrast, the eulophid wasp *T. planipennisi* was consistently found in Beijing and northeast provinces, contributing 2.7–34.4% larval parasitism, but no parasitism by this wasp was observed in Tianjin. In addition, some minor hymenopteran species were also found attacking EAB larvae in the field, such as *Xorides* Latreille (Hymenoptera: Ichneumonidae) species, *Atanycolus* Förster (Braconidae) species, *Sclerodermus pupariae* Yang and Yao (Hymenoptera: Bethylidae), and *Metapelma* Westwood (Hymenoptera: Eupelmidae) species. A parasitic Tachinidae (Diptera) was also found attacking EAB larvae, but with very low parasitism rates (0.4–1.4%).

Coleoptera. In addition to the parasitic wasps, two species of beetles, *Tenerus* Laporte (Coleoptera: Cleridae) species and *X. quadrisignata* Mannerheim (Coleoptera: Trogossitidae), with apparently parasitic larvae, were recovered in the two study sites in Liaoning Province. Parasitism ranging from 1.2% to 21.4% at different sampling times for the Coleoptera (Table 4). To the best of our

Table 2. Prevalence of EAB egg parasitism by *Oobius agrili.*

Sample site	Tree species	Survey dates	Trees sampled (*n*)	EAB eggs (*n*)	Parasitised by *O. agrili* (%)	Other mortality (%)
Dagang, Tianjin	*F. velutina*	2–4 July 2010	10	453	0.00	16.78a
		24 October 2011 to 1 April 2012	10	325	0.00	21.85a
Haidian, Beijing	*F. velutina*	27 October 2010 to 27 May 2011	13	451	3.99b	49.00a
		2 August 2012	12	16	25.00a	18.75b
Fengcheng, Liaoning	*F. mandschurica*	23–24 September 2010	21	103	32.04a	46.60a
		15 July 2011	13	69	31.88a	24.64b
		15–16 May 2012	5	44	4.55b	22.73b
Shangzhi, Heilongjiang	*F. mandschurica*	18 June to 5 August 2012	12	21	19.05	19.05

Notes: Percentages followed by the same lowercase letters in the same column and same site were not significantly different in different years based on likelihood χ^2 test at $\alpha = 0.05$ level.

Table 3. Biotic factors and their suppression against emerald ash borer populations during larval stages.

				Real mortality (%) of EAB larvae caused by*		
Sample site	Survey date	Number sample trees	Number EAB larvae	Parasitoids	Woodpeckers	Others≅
Dagang, Tianjin	27 April to 2 May 2011	10	572	25.00	17.66	17.31
	23 October 2011 to 1 April 2012	13	595	51.60	4.87	18.82
Haidian, Beijing	27 October 2010 to 27 May 2011	13	375	34.13	33.07	10.67
Fengcheng, Liaoning	26–27 September 2008	9	53	45.28	0.00	11.32
	24 September 2010	12	104	2.88	0.00	56.73
	15–16 May 2012	5	162	21.60	0.00	38.89
	16 May 2013	6	186	11.83	0.00	62.37
Shenyang, Liaoning	14 May 2013	7	305	15.74	39.34	7.87
Changchun, Jilin	20–22 September 2008	6	48	31.25	8.33	25.00
Shangzhi, Heilongjiang	18–19 June 2012	7	282	13.83	0.00	47.52

Notes:
* Real mortality is the ratio of the number of dead EAB larvae to the total number of EAB larvae sampled.
≅ Others include diseases, competition, and putative plant resistance.

Table 4. Insect natural enemies of EAB larvae and their apparent parasitism.

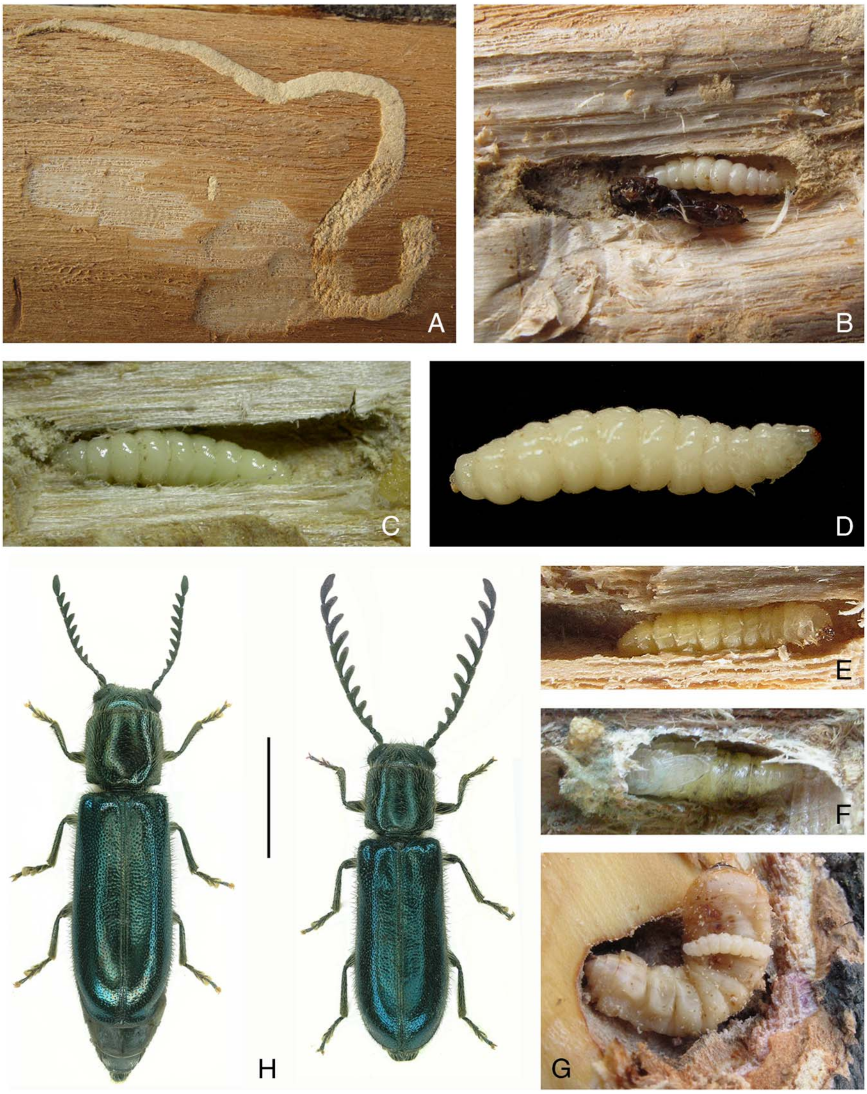
			Apparent parasitism (%) of EAB larvae* by								
Sample site	Sample year	EAB larvae (*n*)	*Tenerus* species	*Xenoglena quadrisignata*	*Xorides* species	*Metapelma* species	*Atanycolus* species	*Tetrastichus planipennisi*	*Spathius agrili*	*Sclerodermus pupariae*
Dagang, Tianjin	April to May 2011	372	0.00	0.00	0.00	0.27	0.00	0.00	36.83	1.34
	October 2011 to April 2012	454	0.00	0.00	0.00	0.00	0.00	0.00	66.74	0.88
Haidian, Beijing	October 2010 to May 2011	211	0.00	0.00	0.00	3.79	5.69	7.11	44.08	0.00
Fengcheng, Liaoning	September 2008	47	19.15	0.00	10.64	0.00	0.00	21.28	0.00	0.00
	September 2010	45	0.00≅	0.00	0.00	0.00	0.00	6.67	0.00	0.00
	May 2012	99	11.11	0.00	1.01	0.00	0.00	23.23	0.00	0.00
	May 2013	70	21.43	0.00	0.00	0.00	0.00	10.00	0.00	0.00
Shenyang, Liaoning	May 2013	161	1.24	1.24	3.73	0.00	0.00	22.98	0.62	0.00
Changchun, Jilin	September 2008	32	0.00	0.00	0.00	0.00	0.00	34.38	12.50	0.00
Shangzhi, Heilongjiang	June 2012	148	0.00	0.00	0.68	0.00	22.97	2.70	0.00	0.00

Notes:

* EAB larvae preyed on by woodpeckers and those that died as young larvae due to other biotic factors were excluded from the larval cohort used to estimate apparent parasitism.

≅ *Tenerus* species was found parasitising a long-horned beetle larvae on Manchurian ash.

Fig. 2. Life stages of *Tenerus* species (Coleoptera: Cleridae). A. Exterior view of EAB pupation chamber; B. larva inside EAB pupation chamber; C. mature larva in EAB pupation chamber; D. Mature larva; E. prepupa; F. pupa; G. larva parasitising a long-horned beetle larva in ash tree; H. adults (left: female; right: male). Scale bar = 2.0 mm.



knowledge, this is the first report of EAB larval parasitism by beetles. Although both beetles are probably predatory as adults, the association of their larvae with single EAB larvae suggests parasitic behaviour.

Larvae of *Tenerus* species were first found in the overwintering chambers of J-shaped mature EAB larvae (Figs. 2A–2D) from girdled Manchurian ash trees in Saima Township, Fengcheng, Liaoning Province in late September 2008. It appears to be a solitary parasitoid of the mature larvae of EAB and the predominant natural enemy during winter in this location, with an average ~13% parasitism (Table 4). We collected 37 mature larvae of this beetle in EAB pupation (overwintering) chambers at the end of September 2008 and 2010, as well as in May 2012 and 2013. When held at room temperature in the laboratory, *Tenerus* species pupated in early August and adults emerged from late August to early September (Figs. 2E, 2F). However, we also collected 13 pre-emergent adult beetles in EAB pupation chambers in field in late

Fig. 3. Life stages of *Xenoglena quadrisignata* Mannerheim (Coleoptera: Trogossitidae); A. pupa inside EAB pupation chamber; B. adult (male).

April 2015, as well as some prepupae and mature larvae. In September 2010, we found *Tenerus* species parasitising larvae of a long-horned beetle (probably *Massicus raddei* (Blessig) Coleoptera: Cerambycidae) on the same host plant (*F. mandschurica*) (Fig. 2G). We collected four long-horned beetle larvae, two of which were parasitised by *Tenerus* species.

Tenerus species has one generation per year in Liaoning Province, China. They appear to over-winter primarily as mature larvae, however, a few immature larvae were also recovered in EAB pupation chambers. The distribution of this beetle was aggregated in the field, as we observed parasitism of EAB larvae by this beetle often occurring on a single tree rather than on many different trees.

The mature larvae are creamy white in colour, spindle shaped (Figs. 2B, 2C), and 8–15 mm in length. Adult beetles are 5.5–7.0 mm long, cylindrical, dark metallic blue with reddish-brown, erect setae. The head and pronotum are covered with dense coarse punctures (Fig. 2H). Antennomere 3 is narrow and triangular, antennomeres 4–10 are strongly pectinate, and the apical antennomere is fusiform.

This newly discovered *Tenerus* beetle is similar to *Tenerus lewisi* Lohde, which is recorded from South Korea and Japan, however host range and biological information of *T. lewisi* are unknown (Kim and Jung 2006). Several morphological characters of this new *Tenerus* beetle differ from *T. lewisi* including: (1) posterior margin of pronotum triundulate with a large median lobe, middle of lobe obviously concave (versus *T. lewisi* posterior margin of pronotum nearly subtruncate); (2) body metallic dark blue (versus *T. lewisi* brilliant metallic blue); (3) antennomere 4 bigger and more prominent (versus *T. lewisi* antennomere 4 smaller and normal) (Fig. 2H).

Xenoglena quadrisignata Mannerheim (Coleoptera: Trogossitidae), the bark-gnawing beetle, was first observed in EAB pupation chambers (Fig. 3A) on *F. velutina* in the Nursery Garden of Shenyang Agricultural University, Shenyang, Liaoning Province, in mid May 2013. Parasitism of EAB larvae by this beetle was very low (< 1%). We observed that *X. quadrisignata* is a solitary parasitoid, attacking mature larvae of EAB in the overwintering chambers, where it develops to the adult stage the following spring. To our knowledge, no literature on its natural history and host records is available. Trogossitids are part of the superfamily Cleroidea (with Cleridae). Our observations of EAB larval parasitism by *X. quadrisignata* appear to represent the first record of a parasitic habit in this family, and parasitism is also rarely seen in the clerids (Clausen 1940).

Xenoglena quadrisignata adults are light grey and ~8.0 mm long (Fig. 3B). The last three antennomeres, eyes, and tarsi are black; all the apical portion of the femora and basal portion of the tibia are dark yellow; while the basal half of the claw is black and the distal half is yellow. The body is elongate, elliptical and flat in shape. The head, pronotum, edge, and median four round points of the elytron are covered with scaly setae. The last three antennomeres are swollen and obviously different from other antennomeres. The entire eye is elliptical and slightly prominent as seen from dorsal view. The anterior margin of the pronotum is U-shaped, surrounding the eyes and posterior head, while the sub-lateral pronotum is concave, the two lateral margins are nearly parallel, and the posterior margins are straight.

Discussion

Field surveys conducted in north and north-eastern China revealed a complex of parasitoids attacking immature EAB. In addition to parasitism, EAB eggs and larvae suffered mortality from other agents such as woodpeckers, disease, and undetermined factors including competition and putative plant resistance. Our survey also indicated that the parasitoid complex and their impacts on EAB mortality varied with different sampling seasons and geographic regions. Liu *et al.* (2003, 2007) previously reported similar findings. For example, the egg parasitoid *O. agrili* and the larval parasitoid *T. planipennisi* were not observed in Tianjin, but these two species were frequently observed attacking EAB eggs or larvae in Beijing, Jilin, Liaoning, and Heilongjiang. On the other hand, *S. agrili* was a major parasitoid of EAB larvae in Tianjin and Beijing. Our results indicate that during the spring season (the late overwintering period), EAB egg parasitism and mortality caused by other factors were lower than that during the late summer. This suggests that mortality by egg parasitoids increases gradually during the adult EAB flight season.

Our study suggests that classical biological control programmes should consider the spatial and temporal variations in the parasitoid complex and parasitism rates in EAB's native range. The affinity of certain parasitoid species for different habitats and possibly climatic conditions highlights the need for post-release monitoring of parasitism levels in North America. The spatial distribution and variability of parasitism across latitudinal ranges within China implies that the egg parasitoid *O. agrili*, as well as the larval parasitoid *T. planipennisi*, are suitable for introduction as biological control agents against EAB in the northern regions (> 40°N latitude) of North America, whereas *S. agrili* would be more appropriate in the southern parts (< 40°N latitude). This may explain why *S. agrili* has not successfully established in Midwest and northeast United States of America (Duan *et al.* 2012a; Bauer *et al.* 2015).

The discovery of two apparently parasitic beetles, *Tenerus* species and *X. quadrisignata*, in association with EAB larvae or prepupae is novel. *Tenerus* species appears to be an important parasitoid of mature EAB larvae or prepupae (~13% parasitism), at least at one study site (Fengcheng, Liaoning Province). In contrast, parasitism of EAB larvae by *X. quadrisignata*, was very low.

Currently, we have little knowledge of the natural history and host range of *Tenerus* species other than contemporaneous observations of attack on larval long-horned beetles. This newly discovered *Tenerus* beetle may have a lifecycle similar to *Dastarcus helophoroides* (Coleoptera: Bothrideridae), a coleopteran parasitoid, which externally parasitise long horned beetle hosts during its larval stage (Wei *et al.* 2009). Although the great majority of species of the family Cleridae are predaceous, a few species appear to develop as external parasites (Clausen 1940). In our study, beetle larvae were found within intact chambers with no visible entrance holes from the bark surface.

Further studies are needed to determine whether these two species of parasitic beetles can be potentially useful for classical biocontrol introduction against EAB in North America, especially for *Tenerus* species because of its relatively higher parasitism in field. Although we surveyed only a limited number of sites within the range of EAB, the presence of novel natural enemies and large variation in mortality among regions suggests that an expanded foreign exploration for EAB natural enemies throughout its native range in Asia may prove valuable.

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