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A New Emerald Ash Borer (Coleoptera: Buprestidae) Parasitoid Species of *Spathius* Nees (Hymenoptera: Braconidae: Doryctinae) From the Russian Far East and South Korea

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ABSTRACT A new emerald ash borer (Coleoptera: Buprestidae) ectoparasitoid species, *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae: Doryctinae), is described from the Russian Far East and South Korea. Molecular evidence supports that Russian and Korean specimens represent a single species and are closely related to two Asian species (*S. agrili* and *S. generosus*) that belong to the *S. exarator* species group. The morphological variation of adult *S. galinae* and its immature stages, distribution, life history, and relation to similar Asian *Spathius* species are discussed. The distribution of the new species may indicate this is one of the more cold hardy emerald ash borer parasitoids. A brief review of hymenopteran parasitoids of emerald ash borer and new host record, the first for *Atanycolus nigriventris* Vojnovskaja-Krieger (Braconidae: Braconinae), is reported.

Резюме Описывается новый вид *Spathius galinae* Belokobylskij & Strazanac – эктопаразитоид изумрудной ясеневой златки *Agrilus planipennis* Fairmaire с юга Дальнего Востока России и Южной Кореи. Молекулярный анализ подтвердил принадлежность российских и корейских экземпляров к одному виду, а также объединение трех азиатских видов (*S. galinae*, *S. agrili* и *S. generosus*) внутри видовой группы *S. exarator*. В статье обсуждаются изменчивость морфологических признаков имаго *S. galinae*, его преимагинальные стадии, распространение, биологические особенности и связь с близкими азиатскими видами рода *Spathius*. Распространение нового вида может указывать на то, что он является одним из наиболее холодоустойчивых паразитоидов изумрудной ясеневой златки. Приводится краткий обзор известных паразитоидов ясеневой златки, а *Atanycolus nigriventris* Vojnovskaja-Krieger (Braconidae: Braconinae) впервые указывается как ее паразитоид.

KEY WORDS *Spathius galinae* n. sp., *Agrilus planipennis*, ectoparasitoid, *Atanycolus nigriventris*, biocontrol

The genus *Spathius* Nees is one of the largest and more polymorphic taxa in the Doryctinae (Belokobylskij 2003). This genus includes ≈300 described species, mostly from the Oriental Region (Nixon 1943, Beloko-

bylskij 2003). The species are divided into nearly 40 species groups, with many species yet to be described, mainly from the Asian continent (Belokobylskij 2003). Species of *Spathius* are known from most temperate and tropical regions of the world, except the Neotropics, where it is replaced by the vicariant genus *Notiospathius* Matthews & Marsh and recently synonymized *Hansonorum* Marsh (De Jesús-Bonilla et al. 2011). Zaldívar-Riverón et al. (2008) in a molecular study of Doryctinae phylogeny indicated members of the tribe Spathiini (including *Spathius*, *Notiospathius*, and *Hansonorum*, among other genera) should be separated into Old World and New World clades.

Most of the species of *Spathius* are idiobiont ectoparasitoids of the xylophagous larvae of numerous genera of several Coleoptera families, including Curculionidae (mostly of Scolytinae), Cerambycidae,

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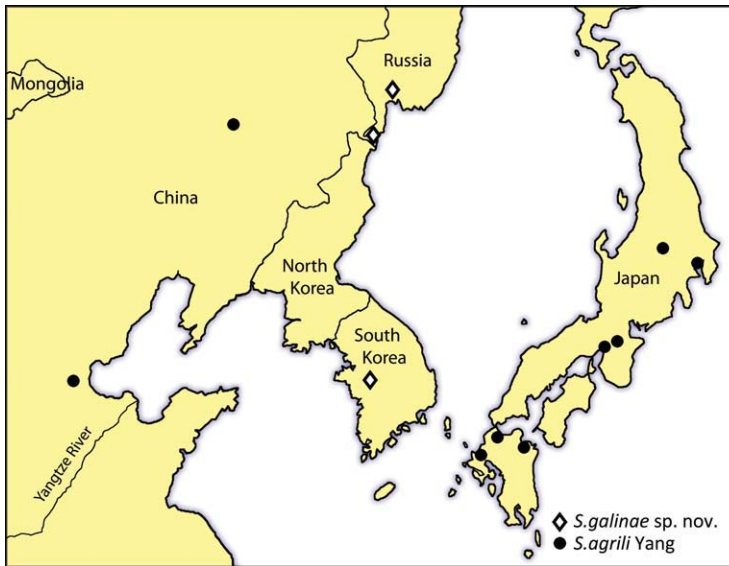


Fig. 1. Collection localities for *S. galinae* sp. nov. and *S. agrili* Yang in Eastern Asia. (Online figure in color.)

Anobiidae, Bostrichidae, and Buprestidae (Shenefelt and Marsh 1976; Belokobylskij 1996a, 1996b, 2003; Yu et al. 2005). *Spathius* species also have been reared from the concealed-living lepidopteran larvae (Sesiidae, Tineidae, Pyralidae, and Tortricidae) and the larvae of xylophagous Xiphydriidae. *Spathius* has been reported to attack cecidophagous Cynipidae, though this needs confirmation.

Larvae of the buprestid *Agrilus planipennis* Fairmaire, the emerald ash borer, feed and develop under the outer bark of ash trees (*Fraxinus* spp.). In their native range, that includes Mongolia (eastern most point), North-Eastern China, the Russian Far East, Korea, Japan, and Southeast Asia, they are considered minor pests of *Fraxinus mandshurica* Rupr., *F. chinensis* Roxb. and *F. rhynchophylla* (Hance) Hemsl. Since the introduction of emerald ash borer into North America (Haack et al. 2002), it has become a major pest of ash, killing nearly all ash trees where it has spread. Poland and McCullough (2006) predicted that emerald ash borer will spread throughout the range of ash in the North America, causing substantial economic and environmental damage.

Another *Spathius* and first braconid parasitoid record of emerald ash borer, *S. agrili* Yang, was found during field surveys to locate natural enemies of emerald ash borer in north-eastern China (Yang et al. 2005). This species has been studied and released in North America as a biocontrol agent of emerald ash borer (Wang et al. 2007, 2010a, 2010b; Strazanac et al. 2009; Yang et al. 2010). Belokobylskij and Maeto (2009) later found *S. agrili* in Japan (Fig. 1). Thus far, extensive surveys for emerald ash borer parasitoids through the south of the Russian Far East have not located a population of *S. agrili* (Yurchenko et al. 2007). During these surveys, an undescribed species of *Spathius* was regularly found attacking emerald ash borer in Primorskiy Krai. It was reported in error as *S.*

depressithorax Belokobylskij (Yurchenko et al. 2007; Baranchikov and Kenis 2008; Baranchikov et al. 2008, 2010; Baranchikov 2010). The distribution and abundance of this new species in climates colder than other known emerald ash borer parasitoids has implications for the biocontrol of emerald ash borer in North America.

This new species of *Spathius* belongs to the *S. exarator* species group as described by Nixon (1943) and partly revised by Belokobylskij (2003). This species group is represented in Eastern Asia by >20 described species from the Russian Far East, China, and Japan. The separation of many of these species requires a combination of morphometric, color, and structural characters (i.e., sculpture and pubescence). The new species is one of these species that require determining multiple character states for it to be differentiated. The complexity of the *S. exarator* species group and limited availability of representative material hinders efficient progress in expanding our knowledge of this group in Eastern Asia. The limitations of the traditional characters used to differentiate the new species efficiently further supports the need for a thorough review of the *S. exarator* species group as it is represented in Eastern Asia.

Materials and Methods

More than 120 specimens of the new species of *Spathius* from the Russian Far East and South Korea (Fig. 1) were examined using an MC-2 ZOOM stereomicroscope (Micromed, St. Petersburg, Russia) and for illustrations. Most specimens were reared from bolts of ash infested with emerald ash borer or directly from emerald ash borer larvae dissected from ash. Adult specimens were also collected on the bark and leaves of emerald ash borer infested ash trees.

Table 1. Specimens used in this study with their localities and GenBank accession numbers

Taxon	Locality	Voucher no.	GenBank accessions	
			COI	28S
<i>Spathius galinae</i> sp. nov.	Russia, Primorskiy Krai, Vladivostok	CNIN657	JN864037	JN864033
<i>Spathius galinae</i> sp. nov.	South Korea, Daejeon	CNIN665	JN864038	JN864034
<i>Spathius galinae</i> sp. nov.	South Korea, Daejeon	CNIN666	JN864039	JN864035
<i>Spathius galinae</i> sp. nov.	South Korea, Daejeon	CNIN667	JN864040	–
<i>Spathius generosus</i> Wilkinson	Russia, Primorskiy Krai, Anisimovka	CNIN654	JN864036	JN864032
<i>Spathius agrili</i> Yang	China, Tianjin, Dagang	EAB-020	AY920299	AY920285
<i>Spathius</i> sp.	Madagascar, Tollara Province	Jo591	AY935476	AY935403
<i>Spathius elegans</i> Matthews	United States	IB67	EF645743	EF645781
<i>Spathius (Stenophasmus)</i> sp.	Uganda, Kibale	Jo675	EF645744	EF645782
<i>Spathius (Stenophasmus)</i> sp.	Madagascar	671005	EF645745	EF645783
<i>Caenophanes</i> sp.	Africa	Jo781	AY935465	DQ498961

Terminology of the morphological features and sculpturing, measurements, and wing venation follow Belokobylskiy and Maeto (2009) and Sharkey and Wharton (1997). The holotype and a series of paratypes were deposited in the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (ZISP). Series of paratypes also were deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, (USNM) and the American Museum of Natural History, New York (AMNH).

We genetically characterized the new described species generating sequences of one mitochondrial (mt) and one nuclear marker. These markers corresponded to ≈ 600 bp of the mt cytochrome oxidase I (COI) gene corresponding to the 'barcoding' locus (Hebert et al. 2003) and ≈ 650 bp of the second and third domain regions of the nuclear ribosomal 28S gene. We also generated one sequence of a specimen of *S. generosus* Wilkinson and included in the analyses previously published sequences of other species of *Spathius*, as well as a sequence of *Caenophanes* to root the COI phenogram. The latter genus appeared as the sister group of *Spathius* in recent molecular phylogenetic studies (Zaldívar-Riverón et al. 2007, 2008). The specimens examined, their voucher numbers, localities, and GenBank accession number are listed in Table 1.

The DNA extraction and amplification procedures are described in Zaldívar-Riverón et al. (2006). COI sequences were obtained using the LepF1 and LepRI primers (Hebert et al. 2004) (LEP-F1: 5'-ATT CAA CCA ATC ATA AAG ATA T-3'; LEP-R1: 5'-TAA ACT TCT GGA TGT CCA AAA A-3'). 28S sequences were obtained with the primers designed by Belshaw and Quicke (1997) (fwd: 5'-GCG AAC AAG TAC CGT GAG GG-3') and Mardulyn and Whitfield (1999) (rev: 5'-TAG TTC ACC ATC TTT CGG GTC CC-3'). Sequences were edited with Sequencher version 4.0.5 (Gene Codes). COI sequences had the same length and 28S sequences only varied by three nucleotides; thus, they could be unambiguously aligned by eye. Genetic distances and number of nucleotidic differences among the specimens examined were calculated separately for the two markers by using the K2P model of evolution with PAUP version 4.0b10 (Swofford 2002). A neighbor joining distance analysis was carried

out for COI by using the above model of evolution. A nonparametric bootstrap (Hillis and Bull 1993) was performed for latter data set by using 1,000 pseudoreplicates and employing a heuristic search, holding one tree per replicate.

Results

Spathius galinae Belokobylskiy and Strazanac, sp. nov.

(Fig. 2-habitus, Fig. 3-illustrations, Fig. 4-head, Fig. 5-mesosoma, Fig. 6-fore and hind wings, Fig. 7-petiole and propodeum, Fig. 8-metasoma, Fig. 9-immature stages)

Spathius depressithorax Belokobylskiy: Yurchenko et al. 2007: 97 (misidentification).

Holotype Female. Body length 4.5 mm; fore wing length 3.3 mm.

Head. Width (dorsal view) 1.4 times its median length (Figs. 3A, B, 4A, C), 1.15 times width of mesoscutum. Vertex convex (Figs. 3C, 4B). Temple (dorsal view) anterior half weakly convex, posterior half roundness decreased; eye transverse diameter (dorsal view) 1.1 times longer than temple. Ocelli with ocellar

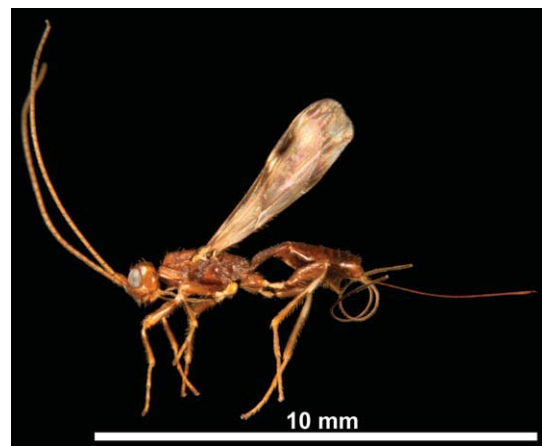


Fig. 2. Habitus of *S. galinae* sp. nov., Russian specimen. (Online figure in color.)

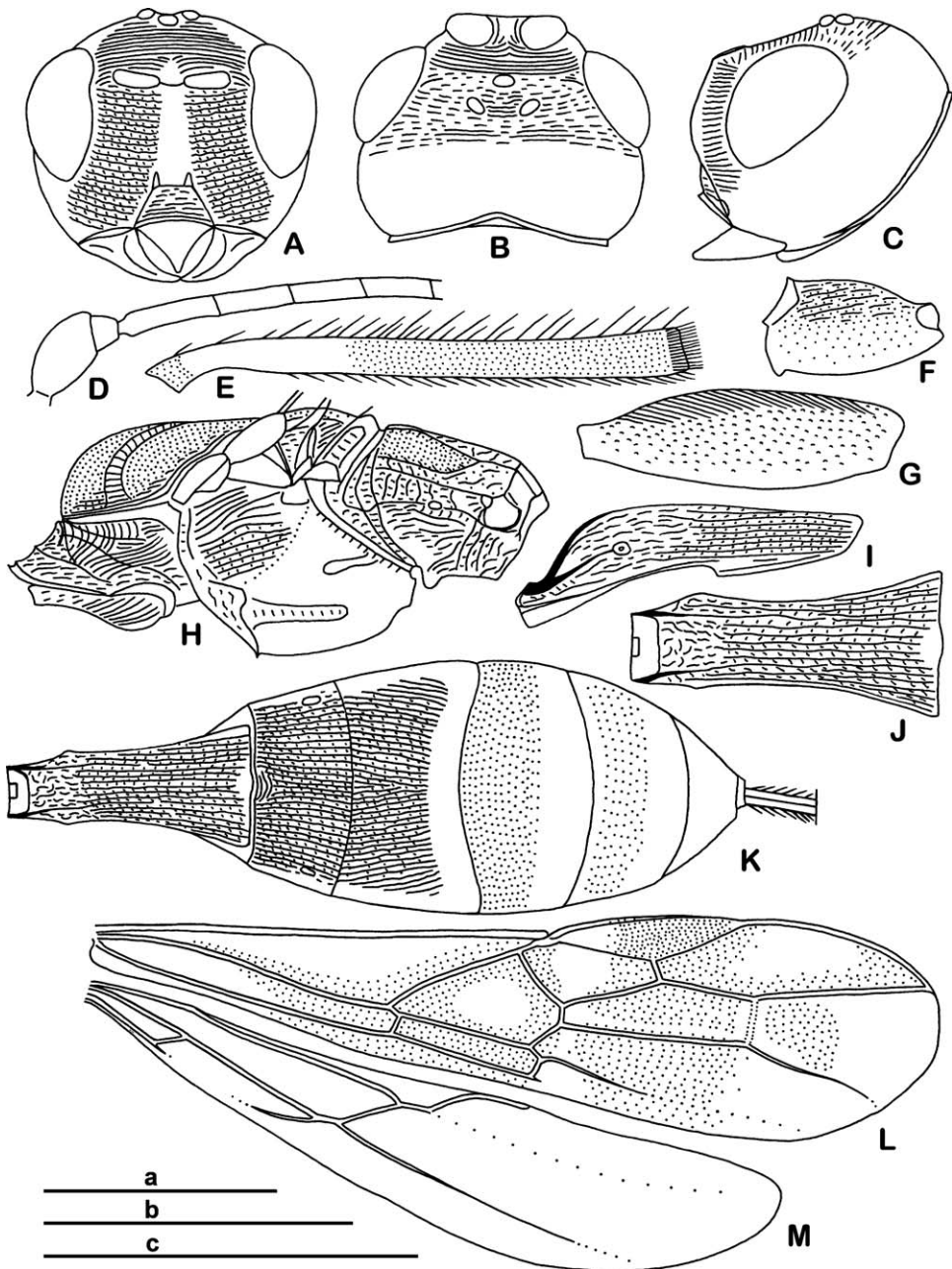


Fig. 3. Structures of *S. galinae* sp. nov. holotype (Russia). (A) Head, anterior view. (B) Head, dorsal view. (C) Head, lateral view. (D) Six basal segments of antenna. (E) Hind tibia. (F) Hind coxa. (G) Hind femur. (H) Mesosoma, lateral view. (I) Petiole, lateral view. (J) Petiole, dorsal view. (K) Metasoma, dorsal view. (L) Fore wing. (M) Hind wing. Scale bars (1 mm): a for L, M; b for H, K; c for A-G, I, J.

triangle base 1.3 times its sides; POL 1.3 times Od, 0.5 times OOL. Eye with very short, sparse setae, its maximum diameter 1.3 times minimum diameter. Malar space height 0.4 times maximum diameter of eye, 0.7 times basal width of mandible. Face convex, its width equal to maximum diameter of eye, 1.1 times height of face and clypeus combined. Clypeal suture deep and complete. Clypeus ventral margin with distinct flange.

Hypoclypeal depression large and rounded, its width 0.9 times the shortest distance from edge to eye, 0.45 times width of face. Occipital carina complete dorsally, not reaching hypostomal carina, terminating before area posterior to the mandible base. Hypostomal flange narrow.

Antennae. Filiform, with >26 flagellomeres (missing apical segments). Scape 1.8 times longer than max-

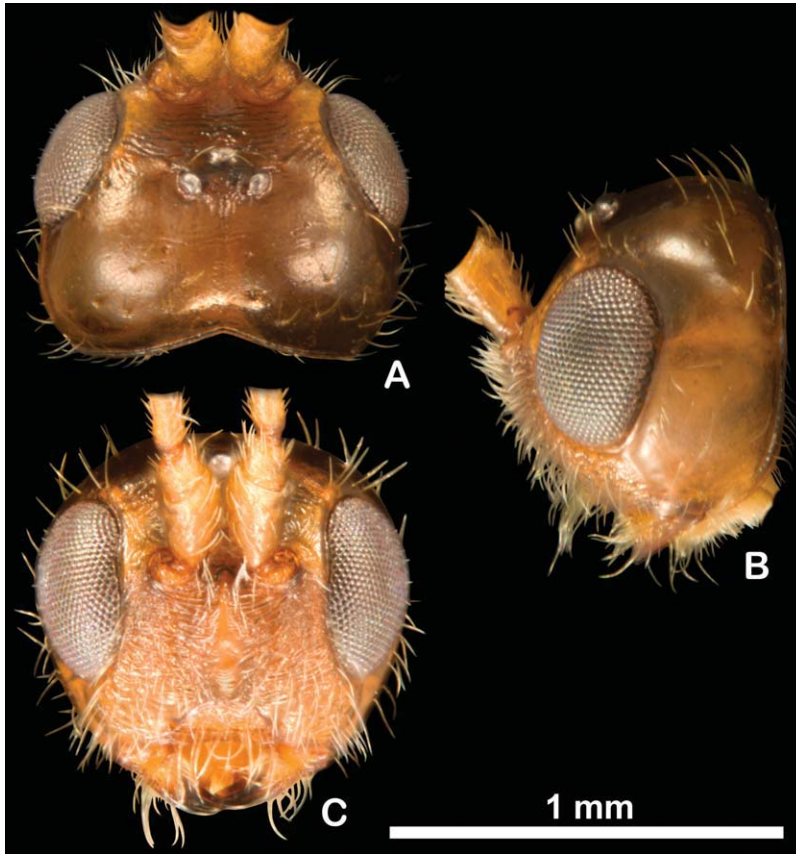


Fig. 4. Head of *S. galinae* sp. nov., Russian specimen. (A) Head, anterior view. (B) Head, dorsal view. (C) Head, lateral view. (Online figure in color.)

imum width (Fig. 3D). First flagellomere 4.0 times longer than its apical width, 1.3 times longer than the second flagellomere. Subapical flagellomeres 2.5 times longer than their width.

Mesosoma. Weakly depressed, maximum length 2.1 times its maximum height (Figs. 3H, 5A, B). Pronotal keel narrow, its posterior branch medially widely fused with posterior margin of pronotum, anterior branch thin and situated submedially. Pronotum (dorsal view) subanteriorly with distinct transverse carina. Pronotal lateral depression distinct, delineated by carinae above and below, narrow anteriorly, widening posteriorly, densely and distinctly crenulate. Mesoscutum (lateral view) anterior rounded and highly elevated above pronotum, its median lobe (dorsal view) convex anteriorly and without anterolateral shoulders. Notauli complete, wide, deep anteriorly, becoming shallow posteriorly, densely rugose-crenulate. Scutellar sulcus (prescutellar depression) shallow, long, with three carinae, densely rugose, 0.3 times as long as scutellum. Scutellum weakly convex, with lateral carinae. Metanotum with short, wide, and pointed apically dorsal tubercle. Subalar depression wide, shallow, densely striate with rugosity. Precoxal sulcus in lower half of the mesopleuron about half its length straight, narrow, shallow, and finely crenulate.

Postpectal carina absent. Metapleural flange (lobe) wide and rounded apically. Propodeum with short and wide lateral tubercles.

Wings. Fore wing 3.7 times longer than wide (Figs. 3L, A). Pterostigma 4.5 times longer than its maximum width. Radial vein (R) arising from middle of pterostigma. Radial (marginal) cell not shortened, metacarp (R1) almost as long as pterostigma. Second radial abscissa (3RSa) 4.3 times longer than first abscissa (R) and forming with it obtuse angle, 0.6 times as long as the weakly curved third abscissa (3RSb), 1.1 times longer than first radiomedial vein (2RS). Second radiomedial (submarginal) cell weakly narrowed distally, its length 3.4 times maximum width, 1.3 times length of brachial (first subdiscal) cell. Second abscissa of medial vein ((RS+M)b) 0.5 times as long as recurrent vein (m-cu). Distance from nervulus (cu-a) to basal vein (1M) 0.3 times nervulus (cu-a) length. Parallel vein (2CUb) not interstitial, arising from anterior third of distal margin of brachial (first subdiscal) cell. Mediocubital vein (M+CU) in distal half weakly curved to longitudinal anal vein (1-1A). Hind wing 4.6 times longer than its maximum width (Figs. 3M, 6B). First costal abscissa (C+Sc+R) 0.6 times as long as second abscissa (Sc+R). First abscissa of mediocubital vein (M+CU) 0.6 times as long as second abscissa (1M). Recurrent vein

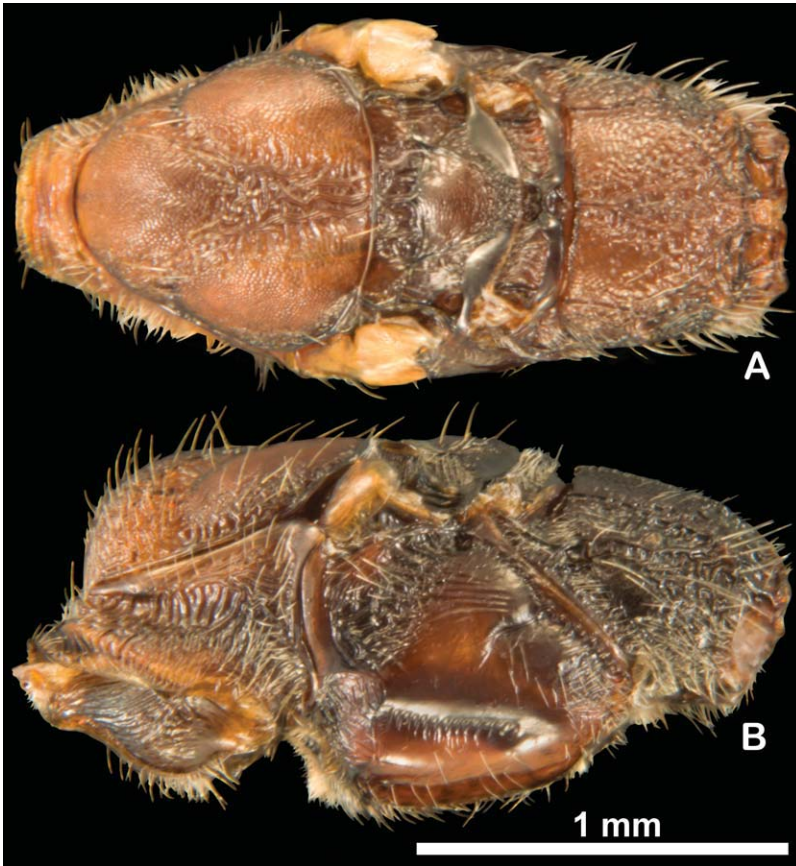


Fig. 5. Mesosoma of *S. galinae* sp. nov., Russian specimen. (A) Dorsal view. (B) Lateral view. (Online figure in color.)

(m-cu) pigmented, distinctly antefurcal, weakly curved, distinctly oblique toward base of wing.

Legs. Fore tibia anterior margin with distinct, closely spaced spines arranged in an irregular row. Segments of middle tarsus much longer than their width. Hind coxa with distinct basoventral tooth, 1.6

times longer than wide (Fig. 3F). Hind femur elongate-oval, 3.3 times longer than wide (Fig. 3G). Hind tibia with outer apical lobe having 4–6 slender spines. Hind tarsus nearly as long as hind tibia (Fig. 2). Hind basitarsus 0.65 times as long as remaining segments combined. Hind tarsus second segment 0.55 times as



Fig. 6. Fore and hind wings of *S. galinae* sp. nov., Russian specimen (A) Fore wing. (B) Hind wing. (Online figure in color.)

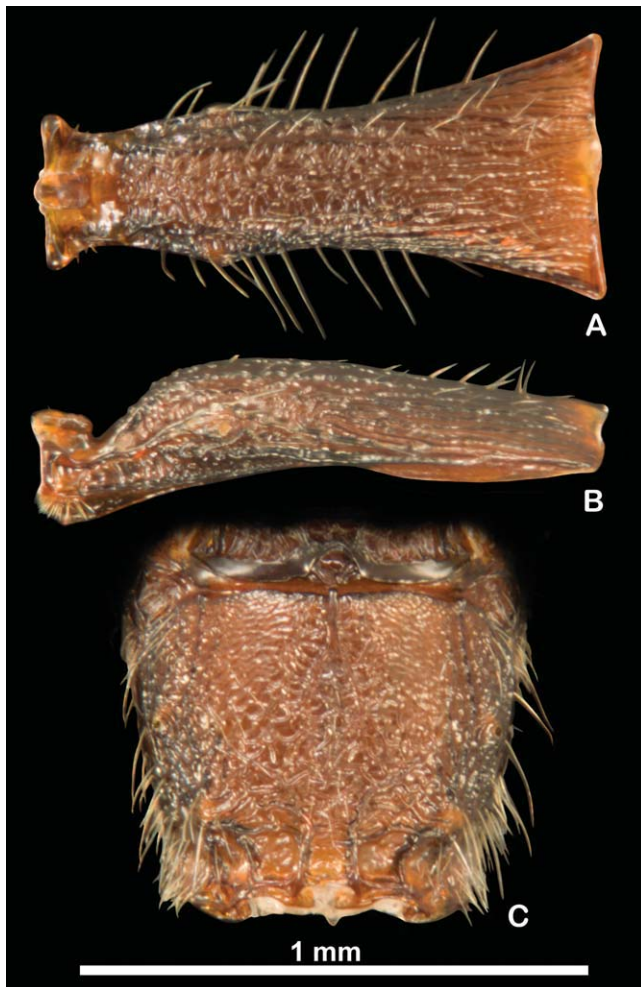


Fig. 7. Petiole and propodeum of *S. galinae* sp. nov., Russian specimen. (A) Petiole, lateral view. (B) Petiole, dorsal view. (C) Propodeum, dorsal view. (Online figure in color.)

long as basitarsus, ≈ 1.6 times longer than fifth segment (without pretarsus).

Metasoma. Petiole (lateral view) ventrally weakly curved upwards, dorsal basal half distinctly arched, apical half nearly straight, highest in basal half (Figs.

3I, 7B); in dorsal view petiole rather stout, widening at spiracular tubercles and apically (Fig. 3J, 7A). Length of petiole 2.0 times its apical width, 1.9 times length of propodeum (Fig. 7C); apical width 1.7 times width at spiracles. Second tergum with laterotergites



Fig. 8. Metasoma of *S. galinae* sp. nov., Russian specimen. (Online figure in color.)

separated. Suture between second and third terga (second suture) very fine and narrow (Figs. 3K, 8). Median length of second tergum 0.6 times its basal width, 0.8 times length of third tergum. Ovipositor slightly curved up (Fig. 2). Ovipositor sheath 3.8 times longer than petiole, 1.4 times longer than metasoma, 2.0 times longer than mesosoma, 0.9 times as long as fore wing.

Sculpture. Vertex mostly smooth, anterior half weakly aciculate-coriaceous (Fig. 3B); frons transverse or curved striate and with fine rugosity between striae. Face mostly striate with fine rugosity between striae, except for a smooth medial narrow vertical area (Figs. 3A, 4C). Temple entirely smooth (Figs. 3C, 4B). Mesoscutum median and lateral lobes granulate, with rugosity along anterior and lateral margins, medioposterior half between notauli with two distinct, undulate and weakly convergent striae, with coarse rugosity between them; lobes near notauli without additional rugae. Scutellum anterior half finely granulate-coriaceous, posterior half granulate with curved transverse striation posteriorly (Fig. 5A). Mesopleuron mostly smooth, distinctly rugose-striate in anterior upper third (Figs. 3H, 5B). Metapleuron anterior half rugulose, posterior half coarsely rugose-reticulate. Propodeum in anterior (dorsal or basolateral) areas lateral to basal (median) carina granulate, other carinae somewhat obscured by strong rugosity (Fig. 7C); basal (median) carina short, 0.45 times as long as anterior fork of (propodeal) areola; areola wide and long, 1.4 times longer than wide; petiolate area short and separated from areola by carina; propodeum densely rugose-reticulate in posterior half. Hind coxa dorsally with curved striae, anterior base rugose, changing to granulate-coriaceous toward apex, posterior and ventral nearly smooth (Fig. 3F). Hind femur finely, densely and obliquely aciculate dorsally, coriaceous laterally, finely coriaceous to almost smooth in lower third (Fig. 3G). Petiole dorsally with dense, weakly undulate striate, rugose-reticulate between striae (Figs. 3J, 7A). Second tergum and basal three quarters of third tergum fine reticulate-striate, and some areolate (Figs. 3K, 8). Fourth tergum basal half fine areolate-punctate; fifth tergum basal half fine and densely punctate, remaining terga smooth.

Pubescence. Vertex with sparse and long semierect setae, glabrous medially (Figs. 4A, B). Mesoscutum mostly glabrous, median lobe of mesoscutum anterolaterally with dense, short, and almost recumbent setae, sparse, long and short nearly erect pale setae arranged linearly along margins of notauli and laterally (Figs. 5A, B). Setae of hind legs semierect, rather long and dense, length of setae on dorsal surface of hind tibia 0.7–1.0 times maximum width of tibia (Fig. 3E).

Color (See Online Color Figures). Head and mesosoma reddish brown, paler ventrally; scutellum, propodeum, metapleuron and metasoma dark reddish brown to black. Antennae yellowish brown to brown in basal half and becoming dark brown to black in apical half. Palpi darkened, brownish. Fore and middle coxae yellowish brown, hind coxa dark reddish brown; all trochanters and trochantelli pale yellow; femora mostly dark reddish brown, fore femur basally and in

apical third, middle and hind femora basally yellowish; tibiae dark reddish brown, fore and middle tibiae basally and apically pale brown to pale yellow at short distances, hind tibia basal two-fifths pale brown to pale yellow; all tarsi light reddish brown to yellowish brown. Ovipositor sheath brown to dark brown. Fore wing distinctly maculate. Pterostigma dark brown, with basal third yellow.

Variation. Body length 2.7–5.6 mm; fore wing length 2.4–4.3 mm. Head width (dorsal view) 1.3–1.4 times its median length, 1.1–1.15 times width of mesoscutum; rounded to depressed dorsally. Vertex widely or entirely smooth, medially and sometimes in anterior third to half finely aciculate to rugulose-striate, most distinctly around ocellar triangle; in large specimens vertex more broadly (on anterior three-quarters in Korean specimens) and distinctly rugulose-striate. Transverse diameter of eye (dorsal view) 1.1–1.3 times longer than temple, but sometimes almost equal to it. Ocellar triangle base 1.2–1.4 times its sides; POL 1.5–1.8 (rarely 1.2) times Od, 0.5–0.7 times OOL. Eye with very short and sparse setae, rarely near bare, its maximum diameter 1.2–1.3 times minimum diameter. Malar space height 0.4–0.5 (rarely 0.3) times maximum diameter of eye and 0.6–0.8 times basal width of mandible. Width of face 1.0–1.1 times maximum diameter of eye, 1.1–1.3 times height of face and clypeus combined. Face median smooth area narrow to wide. Width of hypoclypeal depression 0.7–0.9 times distance from edge of depression to eye, 0.35–0.50 times minimum width of face. Antennae with 27–38 flagellomeres. First flagellomere 4.3–4.6 times longer than its apical width, 1.2–1.3 times longer than second flagellomere. Penultimate flagellomere 2.7–3.3 times longer than width. Apical segment obtuse and without apical “spine”. Mesosoma weakly to not depressed, its maximum length 2.0–2.3 (very rarely 2.4–2.5) times maximum height. Pronotal keel weakly defined to distinct. Pronotal lateral depression more or less distinctly delineated by carinae above and below, densely and distinctly to finely crenulate. Scutellar sulcus (prescutellar depression) with single-three carinae, densely rugose to rugulose. Scutellum entirely densely and finely granulate, sometimes almost smooth in basal third, often with additional transverse striation laterally and posteriorly, rarely in posterior half to two-thirds. Propodeum basal carina 0.5–1.0 (rarely 0.2–0.4) times as long as anterior fork of areola; areola 1.1–1.8 times longer than wide. Fore wing 3.5–3.8 times longer than wide. Pterostigma 4.0–4.8 times longer than its maximum width. Metacarp (R1) 1.0–1.1 times as long as pterostigma. Second radial abscissa (3RSa) 4.0–5.2 times longer than first abscissa (R), 0.6–0.7 times as long as third abscissa (3RSb), 1.1–1.3 times longer than first radiomedial vein (2RS). Length of second radiomedial (submarginal) cell 3.3–3.8 times maximum width, 1.4–1.6 times length of brachial (first subdiscal) cell. Second abscissa of medial vein ((RS+M)b) 0.4–0.6 times as long as recurrent vein (m-cu). Distance from nervulus (cu-a) to basal vein (1M) 0.2–0.5 times nervulus (cu-a) length. Hind wing 4.6–4.8 times longer than its maximum width.

First costal abscissa (C+Sc+R) 0.5–0.6 times as long as second abscissa (Sc+R). Hind coxa 1.4–1.6 times longer than wide. Hind femur 3.1–3.5 times longer than wide. Length of setae on dorsal surface of hind tibia 0.5–0.9 times maximum width of tibia. Hind tarsus 0.9–1.0 times as long as hind tibia. Hind basitarsus 0.65–0.70 times as long as second–fifth segments combined. Second segment of hind tarsus 1.5–1.8 times longer than fifth segment (without pretarsus). Length of petiole 1.6–2.0 (rarely 2.2) times its apical width, 1.7–2.0 times length of propodeum; apical width 1.5–1.7 times width at level of spiracles. Second suture weakly defined to indistinct. Median length of second tergum 0.5–0.7 times its basal width, 1.1–1.3 times length of third tergum. Fourth metasomal tergum basal 0.3–0.4 (rarely 0.2 or 0.5) densely and small reticulate-areolate, rarely finely punctate. Fifth metasomal tergum often smooth basally, but sometimes fine punctation present in its basal third. Ovipositor straight or slightly curved up, its sheath 3.0–4.0 times longer than petiole, 1.2–1.6 times longer than metasoma, 1.4–2.1 times longer than mesosoma, 0.7–1.0 times as long as fore wing.

Color. Head and mesosoma reddish brown to dark reddish brown, usually with darkened dorsal and posterior areas, sometimes all body reddish brown with faint infuscation posteriorly. Antennae basal fourth to half yellowish brown to brown, darkening to near black apically. Femora reddish brown to dark reddish brown, fore and middle tibia basal fifth and hind tibia basal third to half pale yellow to light brown; trochantelli sometimes pale yellow only proximal. Metasoma sometimes yellowish brown in basal half and reddish brown in apical half, rarely entirely dark reddish brown.

Male. Body length 2.8–4.6 mm; fore wing length 2.1–3.4 mm. Head behind eyes often more distinctly convex. Transverse diameter of eye equal to or 1.2 times as long as temple. Antenna with 27–33 flagellomeres. Mesosoma may be distinctly depressed, its length 2.5–2.7 times maximum width. Length of petiole 2.0–2.4 times its apical width. Median length of second tergum 0.8–0.9 times its basal width. Otherwise similar to female.

Immature Stages. *Egg* ($n = 4$) (Figs. 9A, B). Hymenopteriform, spindle-shaped, slender, long, weakly curved, translucent milky white, but transparent marginally, narrowing weakly toward caudal end, and abruptly narrowed (sometimes nose-shaped) toward end of the head; length 1.2–1.4 mm, maximum width 0.13–0.17 mm.

Larva (last stage) ($n = 5$) (Figs. 9C, D). Hymenopteriform, spindle-shaped, with 13 segments and head capsule, the first thoracic segment largest; body entirely covered by very dense and very short setae, narrowing weakly toward head and caudal ends, with single pair of thoracic and eight pairs of abdominal spiracles, cream to pale yellow; length 5.5–6.3 mm, maximum width 1.25–1.40 mm.

Prepupa ($n = 2$) (Fig. 9E). Similar to last-instar larva, slightly contracted.

Pupae of female ($n = 2$) (Fig. 9F). Open, with distinctly separated morphological elements, with separated legs and antenna, ovipositor strongly curved back over metasoma and situated along its dorsal margin, body milky white, eyes dark brown; length without ovipositor 4.0–4.7 mm.

Type Material. HOLOTYPE. Female, Russia, Primorskiy Krai, “Vladivostok, Pr.[ospect] 100-letiya, from *Agrilus planipennis* Fairm. in *Fraxinus pennsylvanica*, col. 2.IV, ex 10.V.2010, G.I. Yurchenko” (ZISP).

Paratypes. RUSSIA: Primorskiy Krai: 12 females, 2 males, labeled as holotype (ZISP, USNM, AMNH); 12 females, 7 males, “Vladivostok, Pr. [ospekt] 100-letiya, from *Agrilus planipennis* Fairm. on *Fraxinus pennsylvanica*, col. I–III.2009, ex. V.2009, N 2, G.I. Yurchenko” (ZISP, USNM, AMNH); 12 females, 4 males, “Vladivostok, pr. [ospekt] 100-letiya, from *Agrilus planipennis* Fairm. in *Fraxinus pennsylvanica*, col. 2.IV, ex. 10.V.2010, N 1, G.I. Yurchenko” (ZISP, USNM, AMNH); 10 females, 10 males, “Vladivostok, from tunnels of *Agrilus planipennis* Fairm. in *Fraxinus pennsylvanica*, ex. 1–3.V.2009, G.I. Yurchenko” (ZISP, USNM, AMNH); 3 females, “Vladivostok, from tunnels of *Agrilus planipennis* Fairm. in *Fraxinus pennsylvanica*, III–V.2009, G.I. Yurchenko” (ZISP); 2 females, “Vladivostok, Borisenko str., on leaves of shoots near dried *Fraxinus pennsylvanica*, col. 11.VII.2010, G.I. Yurchenko” (ZISP); 4 males, “Vladivostok, Postysheva str., on leaves of shoots near dried *Fraxinus pennsylvanica*, col. 24.VIII.2010, N 10, G.I. Yurchenko” (ZISP); 5 females, 2 males, “Vladivostok, Postysheva str., on leaves of shoots near dried *Fraxinus pennsylvanica*, col. 21.VIII.2010, N 7 and 8, G.I. Yurchenko” (ZISP, USNM); 4 females, 5 males, “Vladivostok, Postysheva str., on leaves of shoots near dried *Fraxinus pennsylvanica*, col. 24.VIII.2010, N 9, G.I. Yurchenko” (ZISP); 5 females, “Vladivostok, Postysheva str., on leaves of shoots near dried *Fraxinus pennsylvanica*, col. 15.IX.2010, N 12, G.I. Yurchenko” (ZISP, AMNH); 5 females, “Vladivostok, Postysheva str., on leaves of shoots near dried *Fraxinus pennsylvanica*, col. 14.IX.2010, N 11, G.I. Yurchenko” (ZISP); 2 females, 2 males, “Vladivostok, Ugol'naya st., from *Agrilus planipennis* Fairm. in *Fraxinus pennsylvanica*, 19.VIII.2010, G.I. Yurchenko” (ZISP). SOUTH KOREA: 3 females, 2 males, South Korea: Daejeon, ex. *Argilus planipennis* Fairm., 22.IV.2009, coll. D. W. Williams (ZISP, AMNH); 6 females, 1 male, South Korea: Daejeon, 16.IV.2008, ex *Fraxinus rhynchophylla*, coll. D. W. Williams (ZISP, AMNH).

Etymology. Named in honor of the collector of this species Galina Ivanovna Yurchenko.

Geographic Distribution. Russian Far East (southern Primorskiy Krai) and South Korea (Daejeon) (Fig. 1).

Remark. The specimens from South Korea do not always have the mesosoma distinctly depressed, usually the mesoscutum elevates above the pronotum almost perpendicularly, the vertex is usually distinctly rugulose-striate on its anterior three-quarters, and the body often is dark.

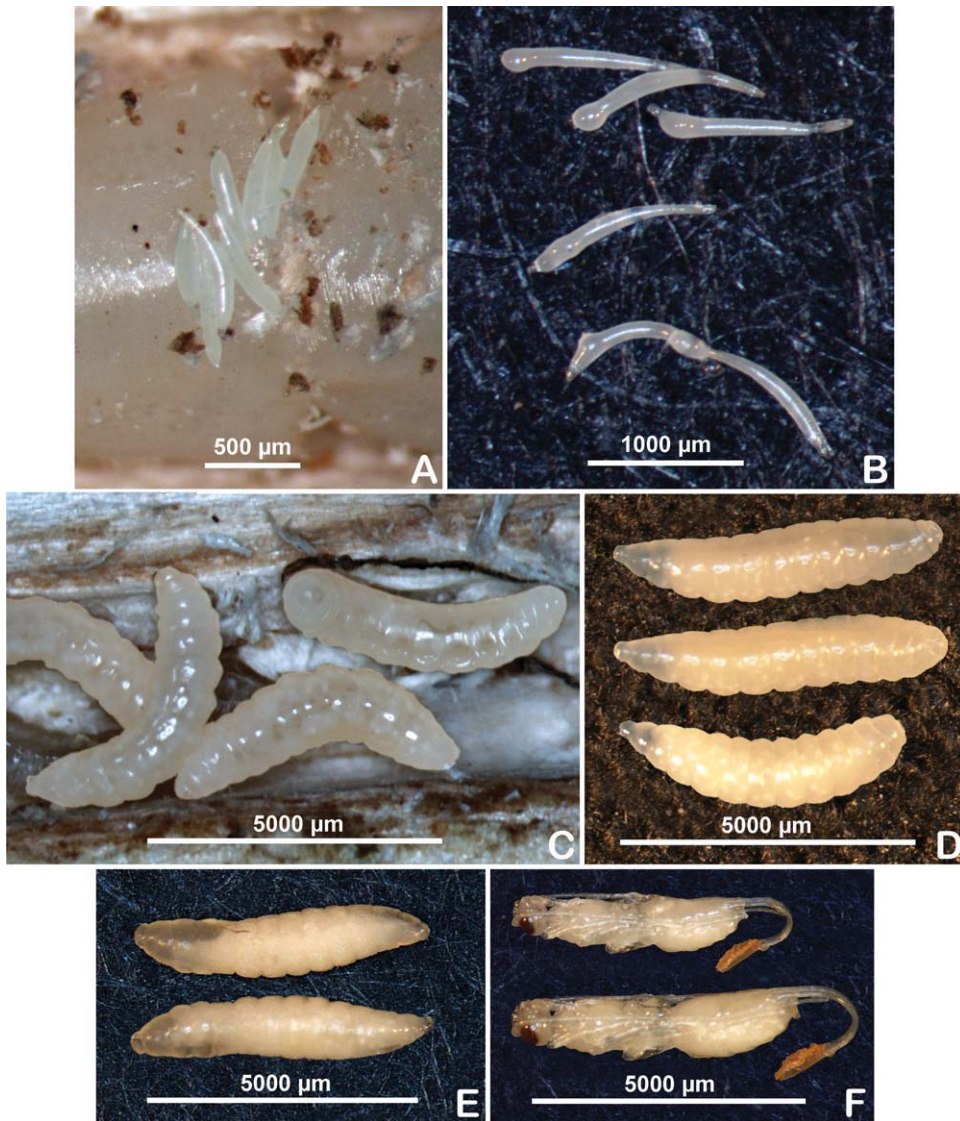


Fig. 9. Immature stages of *S. galinae* sp. nov., Russian specimens. (A) Eggs on emerald ash borer larva. (B) Eggs. (C) Larvae in emerald ash borer gallery. (D) Larvae; (E) Prepupae. (F) Pupae. (Online figure in color.)

Diagnosis. *Spathius galinae* belongs to the *S. exarator* (Linnaeus) species group (see Nixon 1943). This new species is very similar to *S. ussuriensis* Tobias (stat. ressur.) (Tobias 1961, Belokobylskij 1998), but differs in having the ovipositor distinctly longer, 0.7 times to equal the length to the fore wing (0.5–0.7 times fore wing length for *S. ussuriensis*), often more or less curved up (straight in *S. ussuriensis*), the palpi darkened (pale in *S. ussuriensis*), the four tergum usually sculptured basally (usually smooth in *S. ussuriensis*), the hind tibia slender (thickened in *S. ussuriensis*), and the setae on dorsal surface of hind tibia longer (shorter in *S. ussuriensis*). Compared with the other *Spathius* that attacks emerald ash borer, *S. agrili* (Yang et al. 2005), *S. galinae* differs in the hind tibia basally with long pale area (much shorter pale area in *S. agrili*), the

ovipositor longer than metasoma (not longer than metasoma in *S. agrili*), the second tergum long, 1.1–1.3 times longer than third tergum (shorter than third tergum in *S. agrili*), the palpi not as dark, and the fore wing with different pattern of maculation. *S. galinae* also is similar to the Japanese *S. sagaensis* Belokobylskij & Maeto (Belokobylskij and Maeto 2009), but differs in having the head weakly transverse (distinctly transverse in *S. sagaensis*), the transverse diameter of eye 1.1–1.3 times length of temple (1.4–1.5 times in *S. sagaensis*), the hind femur less wide and long (wide and short in *S. sagaensis*), the precoxal suture weakly and narrowly crenulate and mesopleuron without sculpture behind it (widely and coarsely crenulate and mesopleuron with distinct striation behind it in *S. sagaensis*), and the fore wing with different pattern of

maculation. The new species resembles the East Palearctic *S. depressithorax* Belokobylskij (Belokobylskij 1998, 2003; Belokobylskij and Maeto 2009), but differs in having the petiole short and basally strongly arched (long and basally less curved in *S. depressithorax*), the precoxal suture weakly and narrowly crenulate and mesopleuron without sculpture behind it (widely and distinctly crenulate and mesopleuron with distinct sculpture behind it in *S. depressithorax*), the hind tibia basally widely pale (not widely pale in *S. depressithorax*), the second tergum long, 1.1–1.3 times longer than third tergum (shorter than third tergum in *S. depressithorax*), the ovipositor shorter (longer in *S. depressithorax*), the palpi darkened (pale in *S. depressithorax*), and the fore wing with different pattern of maculation. *S. galinae* and the Chinese *S. poecilopterus* Chao from Fujian (Chao 1977) have similar patterns of maculation on the fore wing (holotype at Zoological Institute in Beijing is not available for study). They differ in *S. galinae* having close to half of the basal hind tibia distinctly pale (basal third in *S. poecilopterus*), the fore and middle tibiae mostly dark (pale in *S. poecilopterus*), the second radiomedial (submarginal) cell narrow (wide in *S. poecilopterus*: Fig. 2 in Chao 1977), the second radial abscissa (3RSa) of fore wing slightly longer than first radiomedial vein (2RS) (distinctly longer in *S. poecilopterus*: Fig. 2 in Chao, 1977), the basal (1M) and recurrent (m-cu) veins subparallel (distinctly divergent in *S. poecilopterus*: Fig. 2 in Chao 1977), the first abscissa of basal vein (1RS) short (long in *S. poecilopterus*: Fig. 2 in Chao 1977), the basal carina of propodeum long (short in *S. poecilopterus*), the petiole 1.7–2.0 times longer than propodeum (slightly longer in *S. poecilopterus*), the fifth metasomal tergum often smooth basally (basally with dense and small punctation in *S. poecilopterus*), the ovipositor longer than metasoma (usually shorter in *S. poecilopterus*), and the palpi brown (pale brown in *S. poecilopterus*).

Discussion

Remarks on Taxonomy and Morphology. *Spathius galinae* was reported previously as a parasitoid of emerald ash borer under the name *S. depressithorax* Belokobylskij (Yurchenko et al. 2007; Baranchikov et al. 2008, 2010; Baranchikov and Kenis 2008; Baranchikov 2010). The first available material was a single female (with the label “Primorskiy kray: Khasan Region, env. Ryazanovka, from *Fraxinus rhynchophylla*, coll. 1.10.2005, em. 12.03. 2006, G. Yurchenko”). This specimen lacked host data and its poor condition merited waiting for additional material for species determination, and identified as “*Spathius* (?) *depressithorax* Belok.” by the senior author. Two other specimens with the same labels were preliminary identified as *S. sp. aff. generosus* Wilkinson by the senior author, and later published as *S. generosus* (Baranchikov et al. 2008). Earlier published host records of both *S. depressithorax* and *S. generosus* attacking emerald ash borer based on the preliminary determinations should be disregarded.

Variation of some morphological characters is regularly encountered in this species, including the degree of dorso-ventral depression of the body and the length of the basal carina of the propodeum. A series from a single locality showed variation in the ratio of mesosoma length to height from 2.0 to 2.3, with a few individuals, mostly males, 2.5–2.7. This series also varied in height and shape of mesoscutum upper pronotum in lateral view. The vertex is typically convex, but in some specimens it can be depressed dorsally. Variation in size and degree of body depression may be related to the number of parasitoids that develop on a single host larva and the size and shape of the host’s gallery, as seen in other species of *Spathius* (Belokobylskij 2003: 295).

Distribution, Biology, and Phenology. *Spathius galinae* was first found in 2009 attacking *A. planipennis* larvae in 37- to 40-yr-old *F. pennsylvanica* trees at three sites in Vladivostok (Fig. 1). Adults were reared from ash bolts taken from these sites and placed in rearing containers. These logs were later peeled to collect the cocoons of the emerged adult *S. galinae*. In 2010, adults were again reared in Vladivostok sites and collected from the leaves of ash trees shoots infested with emerald ash borer. Additional surveys produced empty *Spathius*-type cocoons in Primorskiy Krai, including at Ugolnaya, Ussuriysk, Vosdvizhenka, and the MTS FEB RAS Arboretum in Gornotayozhnoe. Exploratory surveys of emerald ash borer and its natural enemies also were conducted in forested areas of Khasan District (2008–2009) that included the native ash hosts of emerald ash borer, *F. rhynchophylla* and *F. mandshurica*. In these stands two *F. rhynchophylla* trees were found to have emerald ash borer galleries (two in one, three in the other) that contained cocoons of *S. galinae* containing pupae, from which adults emerged in September 2010. Exploratory surveys also were conducted in Khabarovskiy Krai in the Khabarovskiy and Nanayskiy Districts. At the Far East Forestry Research Institute arboretum in Khabarovsk, emerald ash borer larvae were found in *F. mandshurica* and *F. pennsylvanica* (three of eleven trees). The density of the emerald ash borer larvae in the *F. pennsylvanica* trees were fairly high, based on larval and exit hole counts (0.22–0.43/dm²), *S. galinae* was not found. Similar results were found for five *F. pennsylvanica* trees from an old nursery for introduced trees and for four trees in Gagarin Park.

S. galinae is a gregarious ectoparasitoid of emerald ash borer larvae, usually associated with third and fourth instars. Typically, 8–12 *S. galinae* larvae are found feeding on a single emerald ash borer larva, with up to as many as 16 found. On a few occasions, one or two parasitoids developed on second-instar larvae. *S. galinae* was found to overwinter most often as prepupae, with pupae and adults found in tunnels early in the spring. Variation in the phenology of emerald ash borer was observed in 2008–2010 that may have affected *S. galinae* development. In April 2009, fourth-instar larvae and pupae of emerald ash borer were collected from peeled *F. pennsylvanica* trees. A considerable number of live *S. galinae* in cocoons were

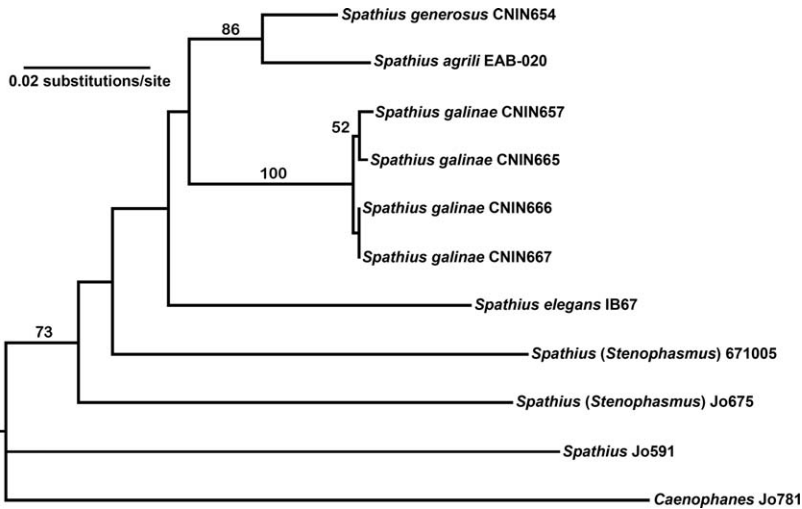


Fig. 10. COI Neighbor joining phenogram (using the k2P evolutionary model) for the specimens of *Spathius* included in the study. Numbers above branches are bootstrap values >50 .

found with them. At the same site in April 2010, most emerald ash borer were dissected out of ash as second-instar larvae and only empty *Spathius* cocoons were found. This suggests the warm September of 2009 allowed the parasitoids to develop over a shorter period than the previous year, and the next generation of emerald ash borer had developed to second-instar larvae. In general, the study sites in Vladivostok had two, and occasionally three generations of *S. galinae* observed per year.

On all study sites in Vladivostok *S. galinae* attacked emerald ash borer larva more intensively on 8- to 18-cm-diameter branches than those with diameters of 5–7 cm. $\approx 50\%$ of emerald ash borer larvae were killed by parasitoids on 18- to 22-cm-diameter ash boles. On ash with larger boles (≥ 36 cm), when 90% of cambium was destroyed by the emerald ash borer galleries, the percent parasitism was lower.

The Russian Far East known population of *S. galinae* lies within an area considered a greater cold hardiness zone than locations where *S. agrili* has been most commonly found and studied in China (Yang et al. 2005, Yang et al. 2010). Based on NAPPFAST (Magarey and Borchert 2007, Magarey et al. 2007), the *S. galinae* are in cold hardiness zones 3–4, and *S. agrili* in Tianjin, China in zone 6. The collecting site for the South Korean *S. galinae* specimens lay in zone 5, near zone 6. Other known *S. agrili* material are from zone 3, though difficult to find there. No *S. agrili* were found in or near Vladivostok in surveys for emerald ash borer parasitoids (Yurchenko et al. 2007). In Japan, *S. agrili* have been widely collected within zones 7–8, and possibly near zone 6.

The degree of cold hardiness of *S. galinae* has potential implications for the biocontrol of emerald ash borer in eastern North America. Our current knowledge of *S. agrili* in Asia is it can be most regularly found in zones 6–8, which in eastern North America covers the middle two-thirds of the United States. Ash is

broadly distributed in eastern North America, reaching its densest levels over large areas in the northern regions (Morin 2010) that are considered zones 3–5. These are the zones *S. galinae* has been commonly found and *A. agrili* less commonly found.

Molecular Results. Our 28S and COI data sets strongly supports that all four examined specimens of *S. galinae* (single female from Primorskiy Krai, Russia and three females from South Korea) are conspecific (Fig. 10). COI genetic distances among the four sequenced specimens of *S. galinae* ranged from 0 to 0.5% (0–3 bp) and were nested in a single clade in the neighbor-joining phenogram generated (bootstrap = 100). The *S. galinae* cluster was separated from the other species of *Spathius* included by genetic distances that ranged from 4.9 to 13.6% (28–78 bp). Between *S. galinae* and the other two known species of *Spathius* that parasitize emerald ash borer, genetic distances were slightly lower with *S. generosus* (0.51–0.53%; 28–31 bp) than with *S. agrili* (0.54–0.58%; 32–33 bp). Within the neighbor-joining phenogram, the three species that attack emerald ash borer form a clade representing the *S. exarator* species group. Three 28S sequences generated for *S. galinae* corresponded to two different genotypes with only one nucleotidic difference between them. Genetic distances for this nuclear marker varied from 1 to 8.7% (6–51 bp) between the specimens of the other *Spathius* that attack emerald ash borer and the remaining species of *Spathius* studied.

Emerald Ash Borer Parasitoid Complex. Since the introduction of emerald ash borer into North America, a taxonomically broad group of hymenopterans has been reported as natural enemies (Duan et al. 2009, Marsh et al. 2009, Kula et al. 2010). Several native North American parasitoids have been identified including *Atanycolus hicoriae* Shenefelt, *A. simplex* Cresson, *A. cappaerti* Marsh & Strazanac, *Spathius florida-nus* Ashmead, *Leluthia astigma* (Ashmead) (all

Braconidae), *Dolichomitus vitticrus* Townes (Ichneumonidae), *Phasgonophora sulcata* Westwood (Chalcididae), and *Eupelmus pini* Taylor (Eupelmidae). The adventive eupelmid parasitoid *Balcha indica* (Mani & Kaul) from Asia also has been regularly recorded (Gibson 2005).

Several Asian parasitoids have been discovered since emerald ash borer's introduction and described, including *Spathius agrili* Yang, *Tetrastichus planipennis* Yang (Eulophidae) (Yang et al. 2006) and *Oobius agrili* Zhang & Huang (Encyrtidae) (Zhang et al. 2005). These three species have since been released in North America (Gould et al. 2010). Other species native to China have been found to attack emerald ash borer, but have received little attention, including *Deuteroxorides orientalis* Uchida (Ichneumonidae), and *Sclerodermus pupariae* Yang & Yao (Bethyidae) (Wang et al. 2009).

Of the three *Atanycolus* native to North America, *A. cappaerti* Marsh & Strazanac, was described as a new species from material that included specimens reared from emerald ash borer (Marsh et al. 2010). The two other *Atanycolus* species reported by Bauer et al. (2008), *A. hicoriae* Shenefelt and *A. simplex* (Cresson), are known to attack a variety of wood boring beetle larvae. The Russian Far East field surveys and subsequent rearings for emerald ash borer parasitoids regularly produced adults of *Atanycolus nigriventris* Vojnovskaja-Krieger, the first host record for this species. Along with *S. galinae*, *A. nigriventris* may represent one of the more cold hardy parasitoids of emerald ash borer.

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