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First Recorded Parasitoid from China of *Agrilus planipennis*: A New Species of *Spathius* (Hymenoptera: Braconidae: Doryctinae)

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ABSTRACT The first reported parasitoid reared from *Agrilus planipennis* Fairmaire, *Spathius agrili* n. sp., is described from China. *S. agrili* was reared from *A. planipennis* in China attacking a *Fraxinus* species native to North America, *Fraxinus velutina* Torr., and one endemic to the region, *Fraxinus mandshurica* Rupr. Life history observations of *S. agrili* in the field and laboratory indicate it is a gregarious idiobiont ectoparasitoid and has up to four generations per year. Parasitism rates in the field ranged from 30 to 90%, with one to 35 *S. agrili* eggs associated with a single host. From a host larva 1–18 adult wasps (average of 8.4) were reared. Based on laboratory rearings, the emerging adult female to male ratio is 3:1.

摘要 本文描述了首次在中国发现的寄生白蜡窄吉丁 (*Agrilus planipennis* Fairmaire) 的寄生蜂一新种——白蜡窄吉丁柄腹茧蜂 *Spathius agrili*. 白蜡窄吉丁在中国主要危害从北美引进的绒毛白蜡 *Fraxinus velutina* Torr. 及中国的本土树种水曲柳 (*Fraxinus mandshurica* Rupr.). 实验室及野外观察表明, 白蜡窄吉丁柄腹茧蜂是群集外寄生于白蜡窄吉丁幼虫, 为抑性寄生习性. 林间每年一般繁殖 4 代. 自然寄生率达 30-90%, 每寄主幼虫上可产 1-35 粒卵, 每头寄主可育出 1－18 头成虫, 平均 8.4 头. 成蜂的雌雄性比为 3:1.

KEY WORDS *Agrilus planipennis*, *Spathius agrili*, new species, parasitoid, biocontrol

Agrilus planipennis Fairmaire (=*A. marcopoli* Obenberger) (Coleoptera: Buprestidae) attacks ash trees (*Fraxinus* spp.) (Oleaceae) in its native range of northern China (including Shandong, Tianjin, Inner Mongolia; Liaoning, Jilin, and Heilongjiang provinces) as well as in Mongolia, the Korean Peninsula, Japan, and the Russian far east. In northern China, it has been a serious pest of imported North American ash [i.e., *Fraxinus americana* L., *Fraxinus velutina* Torr., and *Fraxinus pennsylvanica* varieties *pennsylvanica* Marsh and *lanceolata* (Borkh.) Sarg.-Budd and Best] used in plantations and as ornamentals. It also has been reported attacking native ash [i.e., *Fraxinus mandshurica* Rupr., and *Fraxinus chinensis* varieties *chinensis* Roxb. and *rhynchophylla* (Hance) Hemsl.], most often when associated with the exotic ash species also being attacked. The first major *A. planipennis* outbreaks in China during the early 1960s were on *F. americana*, and they produced great losses for landscaping and timber industries (Yu 1992).

A. planipennis was first collected in southeastern Michigan in May 2002 from ash trees (Haack et al. 2002). It was soon discovered in Windsor, Ontario, Canada, and then Ohio, Maryland (nursery stock from Michigan), and Indiana (Marchant 2004, Rauscher and Mastro 2004). Within southeastern Michigan, it has been reared from all species of native ash and is thought to have been present for at least 5 yr before discovery (McCullough and Roberts 2002). It was predicted that *A. planipennis* could spread throughout the range of ash in North America, causing substantial economic and environmental damage (Haack et al. 2002, Mastro and Reardon 2004). In its native range, *A. planipennis* larvae were reported from species of

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Juglans, *Ulmus*, and *Pterocarya*. However, representatives of these genera in North America have not been reported to serve as hosts.

The concealed *A. planipennis* larvae are difficult to detect until the foliage wilts, dead twigs become noticeable, or adult emergence holes are noted. This delay in detection challenges control efforts with conventional pesticides. The clear threat to ash in North America and China spurred the formation of a Sino-American program to study the potential for biocontrol of *A. planipennis*. During exploration for natural enemies, an undescribed species of *Spathius* Nees (Hymenoptera: Braconidae) was discovered attacking *A. planipennis* larvae in China and preliminary laboratory and field studies of its biology were conducted there. The description of this new *Spathius* species, biological observations, and a discussion of its biocontrol potential are presented.

Typical of most Doryctinae, *Spathius* species attack wood-boring beetle larvae. Worldwide in distribution with >300 species described, *Spathius* is particularly species rich in the Old World tropics (Shenefelt and Marsh 1976) and is distinguished from other winged Doryctinae by 1) forewings with three submarginal cells; 2) first subdiscal cell of forewing closed; and 3) first metasomal segment petiolate, widening apically, lacking basal wing-like projections, and inserted on propodeum near coxal bases (Marsh 1997).

Nixon (1943) studied the Old World *Spathius* species; Shenefelt and Marsh (1976) published a catalog of the world species of *Spathius*. Marsh (1997) produced a key for identification of the genera of New World Doryctinae, including *Spathius*. Matthews (1970) revised the species in America North of Mexico. The Japanese species of the genus were studied by Watanabe (1937). Tobias (1986) reviewed the genus in the European part of Russia and Belokobylskij (1998) described *Spathius* species from Russian Siberia. The Chinese *Spathius* species were studied by the late Prof. Dr. Hsiu-Fu Chao, Fujian Agricultural University, Fuzhou. He listed 40 species for China, of which 34 new species (and one new subspecies) were described by him (Chao 1957, 1977, 1978; Chao and Chen 1965).

Materials and Methods

Surveys during 2003 and 2004 for stressed ash trees were conducted in Tianjin Province, mainly of *F. velutina*, and in Liaoning, Jilin, and Heilongjiang provinces, mainly of *F. mandchurica*. Surveys were performed every other day in 2003 from mid-August to early November and in 2004 from early April to the end of August. Surveys during other months were performed monthly. Trunk bark of stressed trees (in Tianjin, mainly *F. velutina;* and in Liaoning, Jilin, and Heilongjiang, mainly *F. mandchurica*) was peeled back to search for *A. planipennis* larvae and their associated parasitoids. *A. planipennis* larvae were collected and possible parasitoid eggs, larvae, or pupae in cocoons were placed in vials (12 mm in diameter by 75 mm in height). Each vial sample contained a single brood of parasitoids and their *A. planipennis* host larva. These vials were brought into the laboratory, and their contents were transferred into new vials containing a piece of filter paper dipped in distilled water for moisture. The vials were then tightly plugged with sterilized cotton and maintained at 20–23°C in a rearing room. Distilled water was added every 3 d to the filter paper. Parasitoid eggs, larvae, and pupae were successfully reared to adults. After the parasitoids emerged, they were killed and point mounted. Specimens were examined with an Olympus SZH 10 stereomicroscope. Scanning electron microscopy (SEM) photographs were taken with a ZOEL 550LV scanning electron microscope.

Terminology follows van Achterberg (1993). Abbreviations applied to ocelli are OD, longest diameter of ocellus; OOL, shortest distance between a posterior ocellus and compound eye; and POL, shortest distance between hind ocelli. The types are deposited in the Insect Museum, Chinese Academy of Forestry, Beijing, the Nationaal Natuurhistorisch Museum, Leiden, The Netherlands; the U.S. National Museum of Natural History, Washington, DC; and West Virginia University, Morgantown, WV. Authorship of the new species is attributed solely to Zhong-Qi Yang.

As a check on the conspecificity of *Spathius* specimens, we sequenced portions of three genes (16S rRNA, cytochrome oxidase I [COI] and 28S rRNA) from seven specimens arising from three regions: China (Dagang, Tianjin – samples *Spathius*-017 and *Spathius*-020), Japan (Yasu-cho, Shiga – samples *Spathius*-003, *Spathius*-007, and *Spathius*-008), and Russia (two specimens identified as *S. generosus* by Belokobylskij – *Spathius*-050 and *Spathius*-051). Specimens were first photographed using color digital photography (JVC GC-QX5HD mounted on a Leica MZ12.5 stereomicroscope) and environmental SEM (Philips XL30 ESEM-FEG); these photographs are available (along with those of other *Spathius* specimens) on a Web site (Whitfield 2004).

Molecular methodology was as described in Whitfield et al. (2002). Aligned sequences for 16S resulted in a 481-bp data set, for COI 709 bp, and for 28S 691 bp (two of the specimens, *Spathius*-003 and *Spathius*-050, failed to amplify for 28S, so data for this gene are missing), resulting in a total of 1881 bp. Sequences are deposited in GenBank under accession numbers AY920284–AY920302.

Data were analyzed in two ways: 1) maximum parsimony by using PAUP* 4.0 (Swofford 2001), by using a branch-and-bound search and 2) Bayesian analysis by using MrBayes3 (Ronquist and Huelsenbeck 2003), with models selected by MrModeltest 2.0 (Nylander 2002)—F81 for 16S, HKY for 28S, GTR + G for COI—and allowing four chains to sample for 2 million generations (100,000 as burnin).

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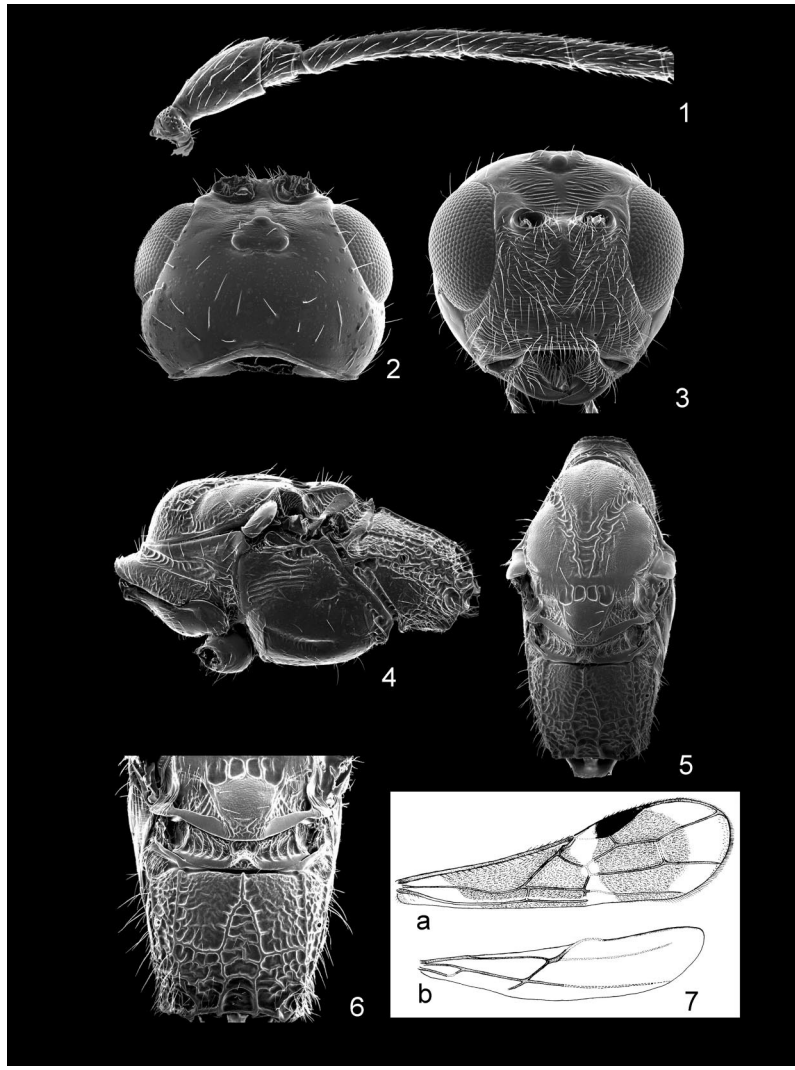


Fig. 1–7. *S. agrili* n. sp. (♀). (1) Antennal segments 1–5. (2) Head dorsal view. (3) Head anterior view. (4) Mesosoma lateral view. (5) Mesosoma dorsal view. (6) Scutellum, metanotum, and propodeum. (7) a, Forewing. b, Hind wing.

Results and Discussion

Spathius agrili Yang, new species

(Figs. 1–14)

Female. Body length 3.5–4.3 mm (holotype 4.0 mm).

Head (Figs. 1–3). Antenna (Fig. 1) 35-segmented, covered with short setae; third segment (first flagellar segment) slightly curved, length 1.6 times the fourth; third, fourth, and penultimate segments with length 6.8, 4.0, and 2.0 times their width, respectively; antenna 1.2 times length of body (without ovipositor). Maxillary palp length 1.5 times height of head. Length of eye in dorsal view 1.4 times temple (Fig. 2). Temple bulging laterally. Occiput slightly concave medially, occipital carina complete; vertex smooth, evenly convex, with few conspicuous short setae. OOL:OD:POL, 9:3:4. Frons medially transversely striate with a longitudinal smooth stripe anterior to median ocellus (Fig. 3). Face evenly convex, with delicate transverse striations on whole surface interrupted medially on upper one-third by a smooth stripe, lower one-third near clypeus superficially rugulose; conspicuous long setae distributed over face and glabrous only below antennal sockets and near malar space. Clypeus convex, finely rugose with anterior margin flanged; width of hypoclypeal depression 0.3 times width of face. Gena smooth; length of malar space 0.8 times basal width of mandible and 0.4 times height of eye in lateral view. Mandible basally with distinctly curved long setae.

Mesosoma (Figs. 4–7). Mesosoma twice as long as high (Fig. 4). Pronotum with lateral upper one-half having about seven transverse rugae and some short longitudinal carinae in a pronotal trough; lower one-half superficially striate. Propleural flange distinct and

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crescentic. Epicnemial carina distinct but upper one-third near anterior margin absent. Precoxal sulcus (sternaulus) extending posteriorly for 0.55 length of mesopleuron, deep, narrow, and weakly crenulate. Epicnemial area concave as a wide, oblique trough with about eight weak transverse carinae; the carina reach the deep episternal scrobe. Mesopleuron medio-posteriorly convex, smooth with a few setae; a deep fovea present between the apices of pleural sulcus and of epicnemial area. Postpectal carina above middle coxa present and extending for some distance. Metapleuron coarsely reticulate, rugose, metapleural furrow deep, complete. Mesoscutum somewhat wider than long (58:54) in dorsal view (Fig. 5), anterior curving strongly downwards to pronotum (Fig. 4). Notauli well defined, converging posteriorly and meeting medially, anterior part (before meeting) crenulate and posterior part (after meeting) irregularly carinate; some setae lateral. Median lobe finely and densely reticulate and lateral lobes with fine reticulation. Scutellum narrows and curves downwards posteriorly (Fig. 5) with length 0.44 times mesoscutum; reticulate, rugose posteriorly; three or four setae along lateral margins. Scutellar sulcus deep with three longitudinal carinae dividing it into four large virtually smooth foveae (Fig. 6). Metanotum with its metascutellum horn-like, protruding medio-posteriorly. Propodeum 0.5 times as long as mesonotum, its median carina short, 0.25 times as long as its branches, transverse, lateral and pleural carinae distinct, transverse carinae arising from the branches of median carina at about half length of propodeum (28:50). Areola large, extending backward to reach area petiolaris at 0.8 length of propodeum, with scattered coarse rugae, dorsal area distinctly (more or less vermiculate) rugose at posterior half, and somewhat finely rugose at anterior half, other areas coarsely and irregularly rugose.

Wings (Fig. 7). Forewing length 2.8–3.3 mm (holotype 3.2 mm). r:3-SR:SR1, 6:18:33; 2-SR:3-SR:r-m, 18:18:11; 1-CU1:2-CU1, 2:27; m-cu:2-SR+M, 8:3; M+CU1 rather strongly sinuate, length of vein r 0.9 times width of pterostigma. Hind wing: 2-SC+R thickened and curved to SC+R1; marginal cell parallel sided from base to apex.

Legs (Figs. 8–10). Hind coxa smooth with a weak dentiform projection baso-ventrally. Length of femur, tibia, and basitarsus of hind leg 3.3, 12.3, and 7.5 times their width, respectively. Fore tibia with irregular row of ≈15 pegs and middle tibia (Fig. 8) with 14 pegs along length of anterior surface. Apex of fore tibia with dentiform projection (Fig. 9). Hind tibia with outer apical lobe having a row of four conspicuous peg-like spines (Fig. 10); hind tarsus as long as hind tibia; length of hind tibial spurs 0.1 and 0.2 times length of hind basitarsus; relative lengths of hind tarsal segments, proximal to distal, 58:29:19:11:17.

Metasoma (Figs. 11–14). Petiolate, as long as head plus mesosoma (Fig. 11), somewhat wider than mesoscutum (34:30). First tergite arched at base in lateral view (Fig. 12), abruptly widening at apex in dorsal view (Fig. 13), its length 2.2 times its apical width, 1.8 times as long as propodeum, 1.3 times middle femur, and 0.8 times the combined length of other tergites, and 1.15 times tergites 2 + 3 (which is as long as tergites 4–7 combined); dorsal carinae extending to hind margin along each side; the dorsum at basal three-quarters coarsely rugulose and apical quarter with fine and longitudinal striae. Tergites 2 + 3 with basal four-fifths distinctly finely granulate to reticulate, the apical fifth and following tergites smooth and shiny. All tergites (except the first) having one transverse row of short setae. Ovipositor bent slightly upwards posteriorly; sheath shorter than metasoma (84:90) (Fig. 14) and 0.6 times as long as forewing.

Color. Reddish brown. Antenna with pedicel and flagellar segments 1–15, trochanters, tarsi, and first tergite yellowish or orange-brown. Palpi and ovipositor sheath brown. Metasoma dark brown except for first tergite. Basal 1/10 of middle and hind tibiae white. Wing veins brown. Forewing infuscate with transverse hyaline bands at base, middle, and apical areas (Fig. 7); vein r-m of forewing and hind wing hyaline.

Variation. The body length among paratypes range from 3.5 to 4.3 mm and forewing length from 2.8 to 3.3 mm. The medial area of the vertex of some individuals has superficial or delicate transverse striae behind posterior ocelli. The hind wing 2-SC+R is thickened and curved to SC+R1 and the marginal cell parallel sided. The anterior third of tergite 4 may be delicately reticulate to granulate. The granulation to reticulation of the anterior two thirds of tergites 2 + 3 may be finely striate.

Male. Body length 3.4–3.8 mm. Forewing length 2.2–2.6 mm. Similar to female except, for the following: antenna 33–37-segmented. Hind wing with 2-SC+R thickened, length 2.7 times its width; length of vein r 0.6–0.9 times width of pterostigma.

Type Material. HOLOTYPE ♀, Dagang, Tianjin City, 5 December 2001, reared from a cocoon collected from galleries of *A. planipennis* in *F. velutina* Torr., Zhong-Qi Yang and Jian-Jun Pang, deposited in Insect Museum, Chinese Academy of Forestry. PARATYPES: 7♀, 3♂, same data as holotype; 23♀, 12♂, 20 June 2002, Zhong-Qi Yang; 35♀, 15♂, 12 August 2002, Xiao-Yi Wang and Zhong-Qi Yang; 26♀, 8♂, 30 May 2004, Xiao-Yi Wang, the other data as holotype; 6♂, Jingyuetan Forest Park, Changchun, Jilin Province, 18 September 2004, Zhong-Qi Yang and Xiao-Yi Wang, reared from the cocoons collected in *A. planipennis* gallery on ash tree *F. mandshurica.* Above-mentioned type specimens are deposited in the Insect Museum, Chinese Academy of Forestry; 10♀, 5♂ (4♀, 1♂, 20 June 2002; 1♀, 4♂, 25 June 2003; 5♀, 15 August 2003), deposited in the Nationaal Natuurhistorisch Museum, Leiden, The Netherlands; 15♀, 5♂, 30 May 2004 in the U.S. National Natural History Museum, Washington, DC; and 4♀, 3♂, 15 June 2004 in the West Virginia University Arthropod Collection, Morgantown, WV.

Distribution. Northern China: Tianjin and Jilin provinces.

Etymology. Specific name derived from genus name of its host, *A. planipennis.*

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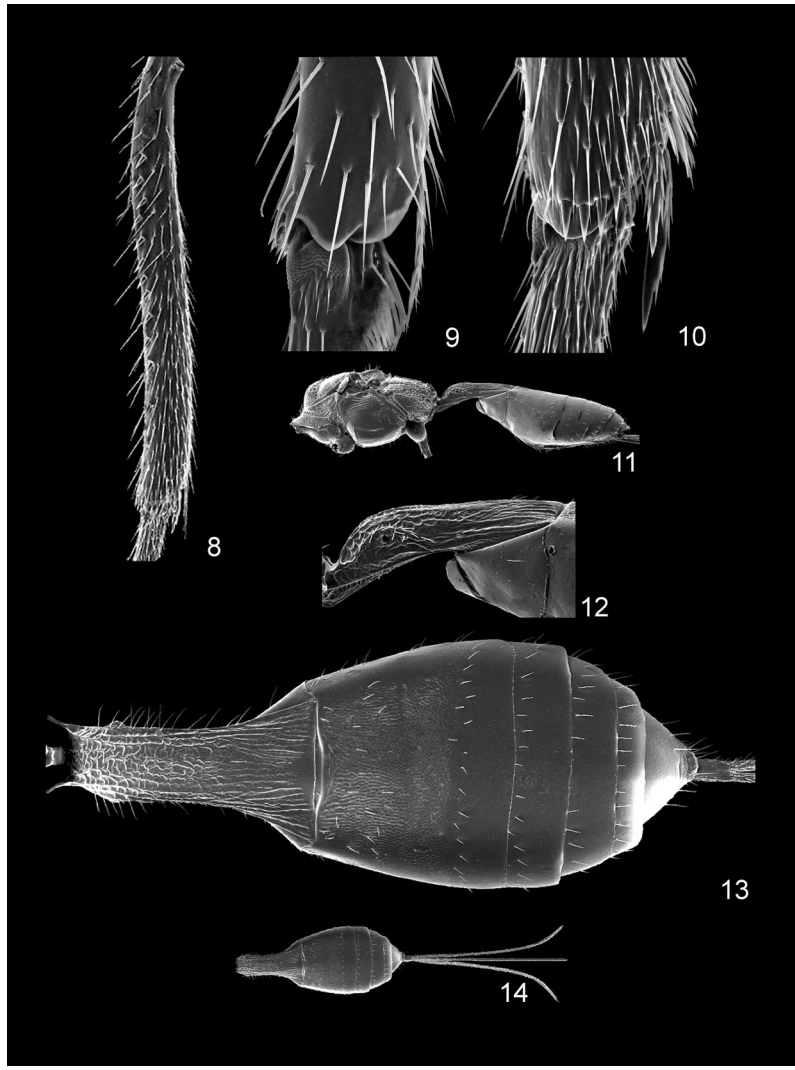


Fig. 8–14. *S. agrili* n. sp. (♀). (8) Middle tibia showing the pegs on anterior edge. (9) Apex of fore tibia showing the dental projection. (10) Apex of hind tibia showing four peg-like spines on apex. (11) Mesosoma and metasoma lateral view. (12) First tergite lateral view. (13) Metasoma dorsal view. (14) Metasoma with ovipositor.

Diagnosis. The new species can be placed in the *S. dissors*-species group (Nixon 1943) and is similar to *Spathius gylippus* Nixon 1943, from the Philippines (*S. gylippus* paratype comparison by J.S.S.). It can be distinguished from the latter as follows: vertex smooth behind ocelli, rarely with weak striation; vein 2-SR of forewing as long as vein 3-SR, basal cell of forewing with a pale band at proximal one-third of wing instead at the median; and vein SR1 of forewing straight (not slightly curved as in the latter). Compared with the European species the new species is similar to *S. erythrocephalus* Wesmael, 1838 (interpretation based on the male lectotype designated by C. van Achterberg and deposited in the Wesmael Collection, Brussels, Belgium). The new species differs by having no distinct subanterior transverse carina on the pronotum; the propodeum rugose; the third and fourth antennal segments somewhat more slender; first tergite orange-brown, contrasting with the dark brown second tergite; length of eye ≈1.4 times temple in dorsal view (about equal in *S. erythrocephalus*); the second tergite comparatively coarsely granulate; and the first discal cell of forewing distinctly paler medially than laterally.

S. A. Belokobylskij (personal communication) considered the new species similar to *S. generosus* Wilkinson 1931, a species reared from *Scolytus major* Strebbing (Coleoptera: Scolytidae) inhabiting *Cedrus deodara* (Don.) (Pinaceae), northern India. The type series (in The Natural History Museum, London, United Kingdom) has been examined by two of us (C.v.A. and P.M.M.) and differs from *S. agrili* by having the first tergite more slender for most of its length, the ovipositor sheath distinctly longer (≈1.3 times as long as metasoma and 0.8 times as long as forewing), basal 0.4 of

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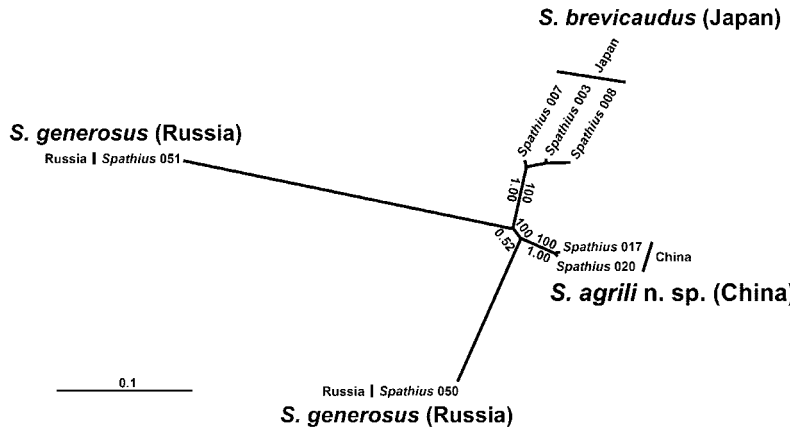


Fig. 15. Tree resulting from both the maximum parsimony and mixed-model Bayesian analyses of 16S, COI, and 28S sequences. Numbers above branches are bootstrap values from the parsimony analysis, and those below are posterior probabilities from the Bayesian analysis. Note that tree is unrooted, because there was no outgroup used.

the hind tibia ivory, vein m-cu of hind wing strongly slanted basad (less strongly so in *S. agrili*), vein r of forewing somewhat shorter (0.2 times as long as vein 3-SR), and the basal areas of the propodeum granulate or superficially rugulose (distinctly rugose in *S. agrili*).

S. agrili is also similar to the holotypes of two Chinese species: *S. nundaensis* Chao 1977 and *S. jilinensis* Chao 1977. These two species compared with *S. agrili* have temples about as long as eyes and are parallel sided, *S. agrili* temples ≈0.7 times as long as eyes and bulging; metasoma as long as ovipositor or shorter, *S. agrili* metasoma 1.1–1.2 length of ovipositor; propodeum between carinae weakly rugolose, *S. agrili* propodeum coarsely rugose; forewings slightly infuscate, *S. agrili* infuscate with three transverse hyaline bands.

From the molecular data analysis, we obtained three most parsimonious trees (treelength, 540; consistency index, 0.98; retention index, 0.94) from the maximum parsimony analyses. A strict consensus tree of three maximum parsimony trees and a tree produced by Bayesian analysis were consistent with the topology shown in Fig. 15.

The two Chinese specimens grouped together (sequences identical for 16S and COI and only differing by 1 bp for 28S), as do the three Japanese specimens (identical sequences for all three genes), but the two Russian specimens are very divergent, *Spathius*-050 grouping relatively near the Chinese samples (but still differing by 25–37 bp for each of 16S and COI), whereas *Spathius*-051 is very separate from the Japanese or Chinese samples (differing by a minimum of 25 bp, usually much greater, from any other cluster). These analyses strongly suggest the presence of at least four species in our seven samples: one from Japan, two from Russia, and one from China that might be somewhat related to one of the Russian samples. Sequence divergences between the four apparent species average ≈5%, well outside the usual range typical of conspecifics, at least for COI (Hebert et al. 2004).

Biological Observations. *S. agrili* is an idiobiont ectoparasitoid, paralyzing the host larva during oviposition and arresting its development. In the field, females were observed to actively search ash tree trunks to locate *A. planipennis* larvae in their sapwood galleries by tapping with their antennae on twigs and trunks. When a host larva was located, the female passed her ovipositor through the bark into the *A. planipennis* gallery and oviposited from one to 35 eggs clustered on the host, which was paralyzed at some point during this process. After wasp larvae hatched they attached to the body surface of the host larva to feed. The larvae consumed an *A. planipennis* larva in 7–10 d. Mature wasp larvae spun individual cocoons gregariously in the end of their host gallery. The parasitoids overwintered as prepupae and pupated near the end of April. *A. planipennis* also overwintered as full-grown larvae in shallow chambers excavated in the outer sapwood and the first adults emerged mid-May. When *S. agrili* adults emerged early June, *A. planipennis* larvae were already present, feeding in beetle galleries. From a single host, one to 18 wasps successfully developed (8.4 adults, on average); the host was killed in all cases. The female to male sex ratio was 3:1. *S. agrili* seems to have up to four generations a year compared with one generation for *A. planipennis.* Parasitism rates reached 30–50% in many forest stands and in a few, 85–90%.

A biocontrol agent should be specific to a target pest and effective in reducing its population to acceptable levels. Presently, we cannot fully address the specificity of *S. agrili*, although in China it seems to be an obligatory parasitoid of *A. planipennis.*

Acknowledgments

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