A New Species of *Laricobius* (Coleoptera: Derodontidae) from Japan with Phylogeny and a Key for Native and Introduced Congeners in North America

Author(s) :M. E. Montgomery, S. Shiyake, N. P. Havill, and R.A.B. Leschen
Source: Annals of the Entomological Society of America, 104(3):389-401. 2011.
Published By: Entomological Society of America
DOI: 10.1603/AN10136
URL: http://www.bioone.org/doi/full/10.1603/AN10136

A New Species of *Laricobius* (Coleoptera: Derodontidae) From Japan With Phylogeny and a Key for Native and Introduced Congeners in North America

M. E. MONTGOMERY,[1,2] S. SHIYAKE,[3] N. P. HAVILL,[1] AND R.A.B. LESCHEN[4]

Ann. Entomol. Soc. Am. 104(3): 389–401 (2011); DOI: 10.1603/AN10136

ABSTRACT *Laricobius osakensis* Montgomery and Shiyake sp. nov., collected from *Adelges tsugae* Annand on hemlock [*Tsuga sieboldii* Carr. and *Tsuga diversifolia* (Maxim.) Mast.] in Japan, is described and illustrated. The new species was collected from several localities on Honshu, Shikokou, and Kyushu Islands. The genus has not been reported previously from Japan. Morphological features, a molecular phylogeny, and diagnostic DNA sites are provided to distinguish this new species from previously described species of the genus. Because of plans to release *L. osakensis* for the biological control of *A. tsugae* in eastern North America, a key and discussion are provided to differentiate it from the native North American species, *Laricobius nigrinus* Fender, *Laricobius laticollis* Fall, and *Laricobius rubidus* LeConte, and from two previously imported species—*Laricobius erichsonii* Rosenhauer, which is endemic in Europe, and *Laricobius kangdingensis* Zilahi-Balogh & Jelínek, which is endemic in China.

KEY WORDS Derodontidae, *Laricobius*, new species, adelgid predators, molecular diagnostics

The family Derodontidae has four genera of small beetles that feed on fungus, except for the genus *Laricobius* Rosenhauer, 1846 (Lawrence and Hlavac 1979, Leschen and Beutel 2010). All *Laricobius* species, with known biology, prey on adelgids (Clark and Brown 1960, Franz 1958, Lawrence and Hlavac 1979). The genus probably occurs throughout the Northern Hemisphere wherever conifers and associated adelgids occur. In 2006, the endemic distributions of *Laricobius* species were as follows: North America (three species); Europe (one) and Asia (seven) (Háva 2006). Since then, eight additional species have been described from Asia—seven from China (Zilahi-Balogh et al. 2007; Háva 2008, 2009a,b,c,d, 2010) and one from Taiwan (Yu and Montgomery 2007).

The Adelgidae are unusual in that they have no known parasites, and predators are the only significant natural enemies. Because members of the genus *Laricobius* specialize on the family Adelgidae, they often are considered as biological control agents for this group. *Laricobius erichsonii* (Rosenhauer) was imported from Europe to control the balsam woolly adelgid, *Adelges piceae* (Ratzeburg); >130,000 were released from 1951 to 1968 in four Canadian provinces and six American states (Clark et al. 1971, Clausen 1978). *L. erichsonii* was believed to have established in all of the provinces and states where it was released; but, to our knowledge, its last recorded recovery occurred in 1978 (Schooley et al. 1984). Recently, the hemlock woolly adelgid, *Adelges tsugae* Annand, has become the target of an intense biological control effort in the eastern United States. This adelgid is native to Asia and western North America but is adventitious in the eastern United States (Havill et al. 2006, 2007). Releases of predators to date for the biological control of *A. tsugae* in the eastern United States consist of lady beetles imported from Japan and China and *L. nigrinus* Fender, which is endemic to western North American (Cheah et al. 2004). The latter has been released in several states in the eastern United States (Mausel et al. 2010). *Laricobius kangdingensis* Zilahi-Balogh & Jelínek has been imported from China, and prerelease studies have been conducted in quarantine (Gatton et al. 2009).

The discovery that the origin of *A. tsugae* introduced to the eastern United States was a lineage in Japan associated with *Tsuga sieboldii* Carrière (Havill et al. 2006) increased efforts to characterize the natural enemies of *A. tsugae* in Japan. As a primary predator of the source population, the new species of *Laricobius* described here is considered a promising candidate for the biological control of *A. tsugae* in the eastern United States. Comparisons are made based on anatomical characters, morphometrics, and mitochondrial DNA sequences to distinguish this new species and other species of the genus that are endemic or previously imported to eastern United States.

[1] Northern Research Station, USDA Forest Service, 51 Mill Pond Rd., Hamden, CT 06514.
[2] Corresponding author, e-mail: michaelemontgomery@fs.fed.us.
[3] Osaka Museum of Natural History, Nagai Park, Osaka 53-0005, Japan.
[4] New Zealand Arthropod Collection, Private Bag 92170, Auckland 1142, New Zealand.

Laricobius rubidus LeConte is the only species in the genus that is endemic to eastern North America. It is a predator of the native pine bark adelgid, *Pineus strobi* (Hartig) but has been found on *A. tsugae* (Montgomery and Lyon 1996; Mausel et al. 2010). Postrelease assessment of impact and spread of *L. nigrinus* has been hampered by the inability to distinguish larvae of *L. nigrinus* and *L. rubidus* based on morphological characteristics (Mausel et al. 2010). Furthermore, although the adults of these species typically are black and reddish, respectively, there are similar looking dark brown adults of both species. The potential addition of a third congener, *Laricobius osakensis* sp. nov., with coloration intermediate of these two species, to eastern North America will compound this problem. Therefore, we used mitochondrial and nuclear DNA sequences to reconstruct phylogenetic relationships among the *Laricobius* species present in North America, plus a few additional species for which we are able to obtain specimens suitable for quality DNA extraction. We also compare sequences from the 5′ end of the mitochondrial cytochrome oxidase I (COI) gene among multiple samples of *L. osakensis* sp. nov., *L. nigrinus*, and *L. rubidus* to determine diagnostic characters that could be used to distinguish native from introduced species. The molecular diagnostics are then compared with more traditional diagnostics based on adult morphological characters.

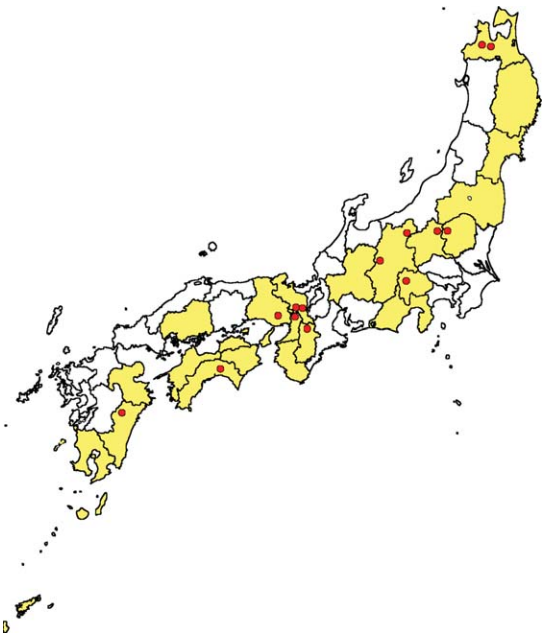

Fig. 1. Range of *L. osakensis* sp. nov. in Japan. Dots, collection localities; shaded areas, prefectures where its host, *A. tsugae* was collected. Hokkaido Island is omitted from the map as it has no natural hemlock and thus was not surveyed. (Online figure in color.)

Materials and Methods

Species Examined. In preparation for the description, 18 of the 19 previously described *Laricobius* species were examined. Fifteen species were types and two of the three species for which the types are lost (*Laricobius caucasicus* Rost and *L. erichsonii*) were represented by specimens collected from the type locality. The only known specimen of *L. minutus* Nikitsky is lost. Additional information about the species can be found in a review of the genus *Laricobius* (Leschen 2011).

The new species of *Laricobius* was obtained from several areas of Japan. We surveyed the Japanese islands of Honshu, Shikoku, Kyushu, and Yakushima and found *A. tsugae* on hemlock at elevations ranging from 50 to 2,000 m in 23 prefectures. The new species described herein was found in 11 prefectures (Fig. 1). Both natural forests and ornamental plantings were sampled throughout the year by beating adelgid infested hemlock foliage over sheets to dislodge adults.

Deposition of Specimens. The museums and their abbreviations where specimens will be placed are as follows: Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, MA; National Museum of Natural History (NMNH), Smithsonian Institution, Washington, D.C., USA; New Zealand Arthropod Collection (NZAC), Auckland New Zealand; Osaka Museum of Natural History (OMNH), Osaka, Japan; and Peabody Museum, Yale University (YPM), New Haven, CT, USA.

Morphology. Whole adult specimens mounted on cardboard points were examined with a stereomicroscope (6–64×). In addition to the new species, we carefully measured 10 examples of five species of special interest: *L. nigrinus*, *L. rubidus*, *L. laticollis*, *L. erichsonii*, and *L. kangdingensis*. The width and length of the head, prothorax, and elytra were made using an eyepiece ocular in a Wild microscope at 50× (1 unit = 0.02 mm). The number in a row and per millimeter of elytral punctures were counted in the scutellary striole and the third complete row. The diameter and distance between punctures of the elytral striae were imaged and measured with a VHX digital microscope (Keyence, Osaka, Japan). Antennae, genitalia, and other parts were cleared and mounted on slides for viewing with a compound microscope. A digital camera mounted on a third tube on the microscopes was used to record the images. A microscale was photographed at each magnification and incorporated into the raw image. Helicon Focus Pro (Helicon Soft Ltd., Kharkov, Ukraine) was used to provide increased depth of field of habitus images. Type and direction of lighting is extremely important because of the shiny surfaces and deep pits and punctures characteristic of these species. Usually, a fiber optic ring light was used, with various portions masked to bring out details.

Morphological presentation follows Franz (1958) with terminology modernized after Lawrence et al. (2010). The external morphology of the ventral thoracic surfaces was compared with the example of *L. nigrinus* in Ge et al. (2007).

Molecular Phylogeny. DNA was extracted from beetle abdomens using the DNA IQ extraction kit (Promega, Madison, WI). The remaining head and

Table 1. Specimens used for molecular phylogeny

Species	Collecting information	Voucher no.	GenBank accessions			
			COI	EF1μ	*wingless*	ITS2
Peltastica tuberculata Mannerheim	USA; Washington; Jefferson County; Salmon Cr. PC; 17 Oct. 2008; G. Kohler	YPM716221	HQ433479	HQ433492	HQ433501	N.A.[a]
Derodontus maculatus (Melsheimer)	USA; Georgia; Clarke County; Athens; nr. Memorial Park; 13 Jan. 2008; J. V. McHugh, T. R. McHugh	YPM716231	HQ433480	HQ433493	HQ433502	N.A.
Laricobius caucasicus Rost	GEORGIA; Guria Province; Bakhmaro; 2,000 m; 9 June 2007; M. Kenis	YPM716730	HQ433481	HQ433494	HQ433503	HQ433485
Laricobius erichsonii Rosenhauer	United Kingdom; Berkshire County; Dinton Pastures; 14 July 2005; P. M. Hammond	BMNH675578	HQ433482	HQ433495	HQ433504	HQ433486
Laricobius kangdingensis Zilahi-Balogh & Jelínek	China; Sichuan Prov.; Lixian County; 27 Sept. 2007; Jinhua Zhou	YPM716321	HQ433483	HQ433496	HQ433505	HQ433487
Laricobius laticollis Fall	USA; Washington; King County; Seattle; 17–24 Feb. 2007; R. McDonald, T. Coleman	YPM716728	HQ433484	HQ433497	HQ433506	HQ433488
Laricobius nigrinus Fender	USA; Idaho; Kootenai County; Coeur d'Alene; Couger Gulch; 9 Mar. 2007; D. Mausel	YPM716517	HM803412	HQ433498	HQ433507	HQ433489
Laricobius osakensis sp. nov.	Japan; Osaka Prefecture; Nov. 2006; A. Lamb	YPM716233	HM803300	HQ433499	HQ433508	HQ433490
Laricobius rubidus LeConte	USA; Connecticut; Hamden; Ingram Street; 27 Mar. 2007; N. Havill	YPM716539	HM803434	HQ433500	HQ433509	HQ433491

[a] N.A., not applicable, refers to sequences not used in this study because alignment with other taxa was ambiguous.

thorax on paper points and slide-mounted genitalia of males were deposited at YPM. Representatives of two other derodontid genera, *Peltastica tuberculata* Mannerheim and *Derodontus maculatus* (Melsheimer), 1844, were included as outgroups (Table 1). The 5′ end of COI was amplified and sequenced using the primers LepF1 and LepR1 (Hebert et al. 2004). Elongation factor-1α (EF1α) was amplified using a heminested polymerase chain reaction (PCR). Overlapping regions were amplified and sequenced with primers For1deg and EFA5, and EFS372 and Cho10Rev1, by using product from an initial PCR that used primers For1deg and Cho10rev1 (McKenna et al. 2009). Partial *wingless* was amplified and sequenced with primers Wg578 F and WgAbR (Wild and Maddison 2008). Internal transcribed spacer (ITS)2 was amplified and sequenced using primers ITS3 and ITS4 (White et al. 1990). Sequencing reactions were performed using the BigDye Terminator kit (Applied Biosystems, Foster City, CA) and analyzed on a 3730 automated sequencer (Applied Biosystems). Sequences were edited using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, MI) and aligned using MUSCLE 3.6 (Edgar 2004). Sequences have been deposited in GenBank (Table 1). Partition homogeneity tests were performed with 1,000 replicates as implemented in Paup* 4.0b10 (Swofford 2003) for each pair of genes. Maximum parsimony analysis was conducted for the combined data set using PAUP* with random sequence addition, tree-bisection-reconnection branch swapping, collapsing zero-length branches, and equal weighting of characters. Maximum parsimony clade support was estimated using 1,000 bootstrap replicates with the same search conditions. Bayesian analysis was conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) by using default priors, four incrementally heated Markov chains, and two concurrent runs of 1,000,000 generations sampled every 1,000 generations. After examining the plot of log-likelihood versus generation to assess stabilization of Markov chains, the first 100 trees were discarded, leaving the stable trees. The substitution model for each region was determined using AIC as implemented by Modeltest 3.7 (Posada and Crandall 1998).

Molecular Diagnostics. DNA was extracted and COI was amplified and sequenced as described above. Sequences were obtained from 25 *L. osakensis* sp. nov. individuals (21 unique haplotypes), 114 *L. nigrinus* (28 haplotypes), and 213 *L. rubidus* (58 haplotypes). Vouchers of each specimen (minus the abdomen) were deposited at Yale University's Peabody. All sequences were deposited in GenBank (accessions HM803282–HM803687). Intra- and interspecific variation was evaluated using Kimura two-parameter (K2P) distances calculated using PAUP*. The CAOS software P-GNOME (Sarkar et al. 2008) was used to identify diagnostic characters in COI sequences to distinguish *L. osakensis* sp. nov. from *L. nigrinus* and *L. rubidus.* A neighbor joining reference tree of unique haplotypes was generated in PAUP* and manipulated in MacClade 4.08 (Maddison and Maddison 2005) to form collapsed clades for each taxon of interest as described in the CAOS manual. This tree was used as a guide by the software to identify diagnostic characters to distinguish taxa.

Laricobius osakensis Montgomery & Shiyake, sp. nov

(Fig. 2-habitus, Fig. 3-parts, Fig. 4A-male genitalia)

Diagnosis. The absence of ocelli on the head distinguishes this species from all other *Laricobius* species examined, except *Laricobius taiwanensis* Yu & Montgomery, and *L. kangdingensis.* Compared with these two species, the frons of this species is smoother,

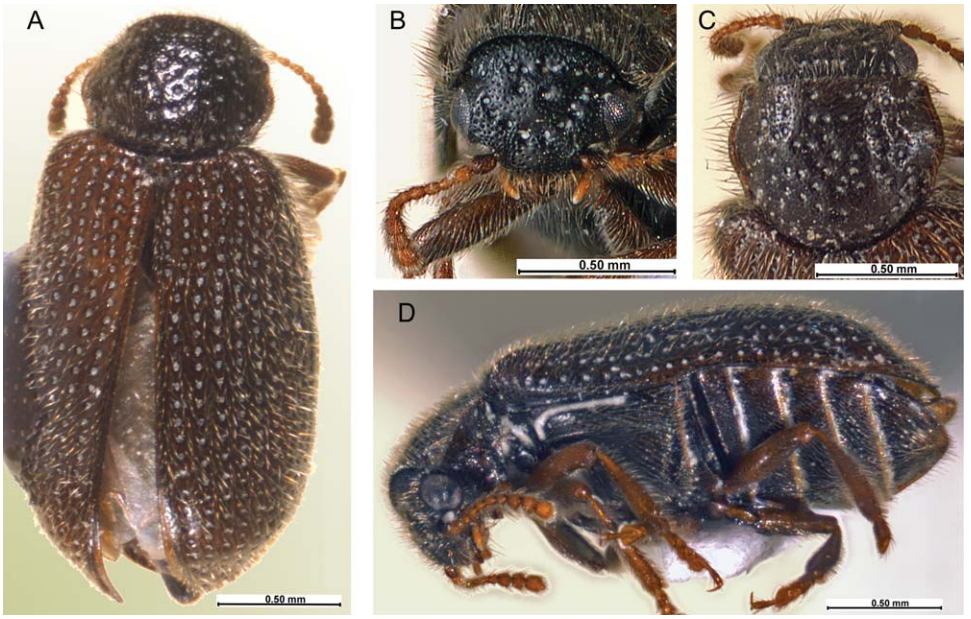

Fig. 2. Habitus of *L. osakensis* sp. nov. (A) Dorsal view. (B) Head. (C) Prothorax. (D) Ventral-lateral view. (Online figure in color.)

with the central area not bulged or bordered by deep irregular invaginations; the posterior angles of the pronotum are rounded, without a small tooth; and the elytra has punctures that are larger, with distance between striae two-thirds puncture diameter rather than ≈1 diameter.

Male. Body elongate, dorsally convex, ventrally flattened, length 3.1 mm (2.9–3.4), width 1.25 mm (1.22–1.28) (Fig. 2A and D). Color of head and thorax black, remainder various shades of brown. Dorsal surface with deep, slightly countersunk punctures penetrating almost through integument, diameter 0.04–0.05 mm (approximate width of sixth antennal segment); punctures contain whitish secretion (Fig. 2), or empty (Fig. 3E). Micropunctures, approximately one-third the size of the deep punctures and penetrating only the cuticle, are on all ventral and dorsal body surfaces, except the elytra. Vestiture of fine, semierect hairs of length 2–3× diameter of punctures.

Head oblong-ovate, 1.5 wider than long, partially concealed by prothorax (Fig. 2B). Eyes large, convex, finely facetted. Ocelli absent. Deep, setose cavity between eye and antenna. Frons relatively flat, with approximately eight round, deep punctures forming a rough U starting at mid-level of each eye and ending just above frontoclypeal area, additional two to four punctures at upper center of frons. Finer micropunctures dispersed more densely and evenly on frons and clypeus. Occipital region with well-developed transverse grove. The area posterior to the grove sculpted with very shallow oblong micropuncutes perpendicular to the grove, ≈2× length of micropunctures on frons. Frontoclypeal area slightly raised, without transverse impression between frons and clypeus. Clypeus short, length one-half diameter of eye, anterior edge slightly convex.

Antennae (Fig. 3A) slender with 11 segments; segments I and XI longest and subequal, segments II and III subequal, segments IV–XII subequal, segment XIII shortest and equal to width, last three segments are much broader than others forming a weak club, apex of terminal segment asymmetrical.

Prothorax (Fig. 2C) wider than head, narrower than combined width of the elytra, 1.3× wider than long, widest at midlength, anterior and posterior widths approximately equal. Dorsal surface with a large fovea mesal of each anterior and posterior angle, 60–70 randomly scattered deep punctures, approximately the same size as head, micropunctures 3–5× more numerous, evenly distributed, well formed. Disc transversely convex, less so at midlength; lateral edge explanate, bordered by a rim. In outline, anterior margin nearly straight, forming slightly obtuse angle with lateral margin, lateral edge arcuate, rounded at posterior with a small sharp angle inward, joining with the evenly convex posterior margin. Lateral pronotal carina separating disc and hypomeron is well developed and acute.

Scutellum color same as pronotum and sutural line; shield shaped; hairs whiter, denser than on pronotum or elytra.

Elytra brown (Fig. 2A) (varies from tan to dark brown), faint darker maculation near suture and lateral edge. Elytra length 1.86 mm and 1.54× (1.37–1.66) width of both elytra, elytral length to pronotum length is 3.2× (2.8–3.2). Elytral base with slightly raised, rugose shoulder and feeble, transverse impression at one-fourths length from base. Scutellary striole consisting of nine (six to 10) punctures. Ten striae present,

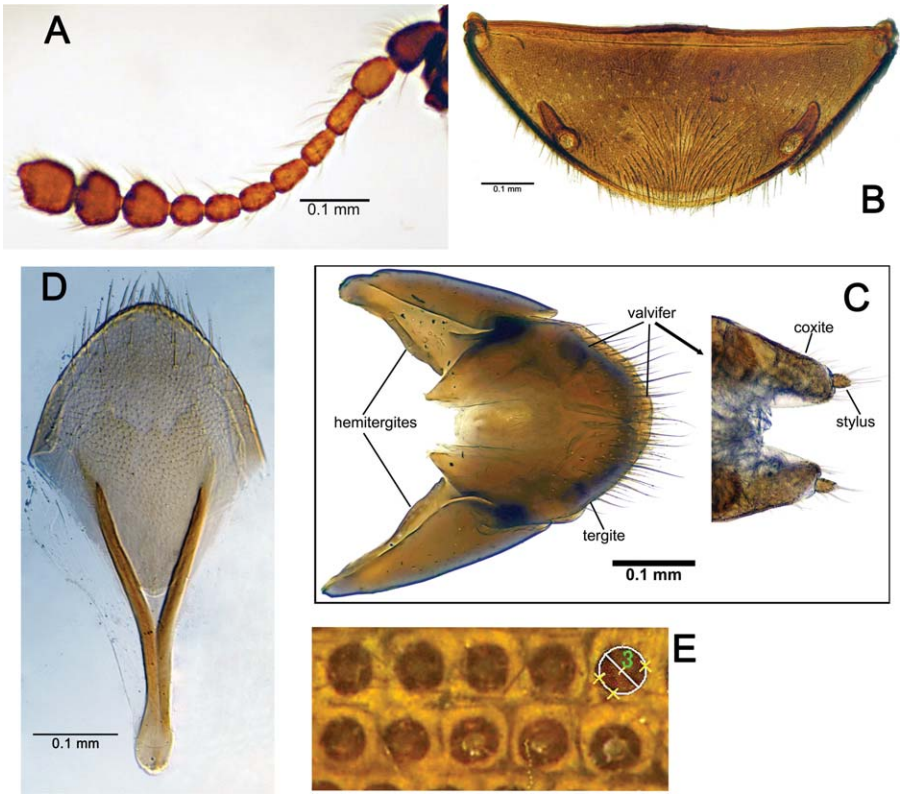

Fig. 3. Structures of *L. osakensis* sp. nov. (A) Antenna. (B) Female seventh abdominal sterite. (C) Female genitalia, dorsal view, ninth segment with hemitergites (pleurites of Franz 1958) and tergite, valvifers (ninth sternite) are partially visible underneath and shown extruded to display the paired coxites and terminal styli. (D) Male ninth abdominal sternite. (E) Detail of elytral punctures in middle of rows 3 and 4, diameter of puncture labeled "3" is 0.05 mm. (Online figure in color.)

stria III with 30 punctures (27–33); average diameter 0.054 mm (0.050–0.056), interstrial distance between punctures 0.037 mm (0.33–0.039). Striae not incised, except near epipleuron and striole and humerus. Between punctures, cuticle smooth with faint microsulpture suggested by reflected light; rectilinear lines visible beneath cuticle with strong, transaxial light (Fig. 3E).

Legs dark, nearly same color as ventral segments, slender; femur and tibia subequal in length and color; tarsi with five joints, proximal three enlarged, lobed, fourth small, fifth as long as I–III combined; claws simple.

Ventral surfaces including anterior portion of epipleuron dark chocolate brown to black, anterior and lateral portions of abdominal segments usually lighter. Micropunctures clearly visible on sterna and abdominal segments. Procoxal cavities open, mesocoxal cavity rounded, intercoxal process separated in middle third. Metaventrite with faint discriminal line in lower one third, katepisternum divided by distinct cleft. Epipleuron distinct to level of posterior edge of metaventrite. Abdomen with five visible sternites, segment 2 concealed by coxae, 3 and 4 connate, ventrite 5 with lateral fovea (Fig. 3B).

Aedeagus (Plate 2A). Total length 0.50 mm, one-sixth body length. Phallobase length/width = 0.56, emarginate at base, with median carina two-thirds length. Median lobe 2.25× longer than basal piece, extending beyond apex of parameres, gradually tapering from base to oriface, with subacute apex. Parameres 1.5× longer than basal piece long; slender, width at midlength approximately one-third width of the median lobe; curving weakly inward, tapering to sharp apex. Male ninth sternite (spiculum gastrale) apex narrow, acutely rounded (Fig. 3D).

Female. With 80% of the specimens, the elytrae of females differed from males in being bicolored, with a lighter, more tannish base color and darker, more extensive maculation along the lateral edge three to four striae wide in anterior portion. Female genitalia (Fig. 3C, dorsal view) have the tergite of the ninth segment with apex broadly rounded; beneath is the ninth sternite (valvifers), which are also shown protracted to show the pair of two-segmented appendages, coxites terminated by small styli. Sensory hairs and pores are abundant on the apical portions of the sternites and tergites.

Type Data. In addition to the collection information label, all specimens have a yellow label (red for holotype) with the type designation in capital letters and *Laricobius osakensis*, Montgomery & Shiyake. Many specimens also have a separate label with a number to link DNA results; e.g., Havill sample 09-79.3 (only the

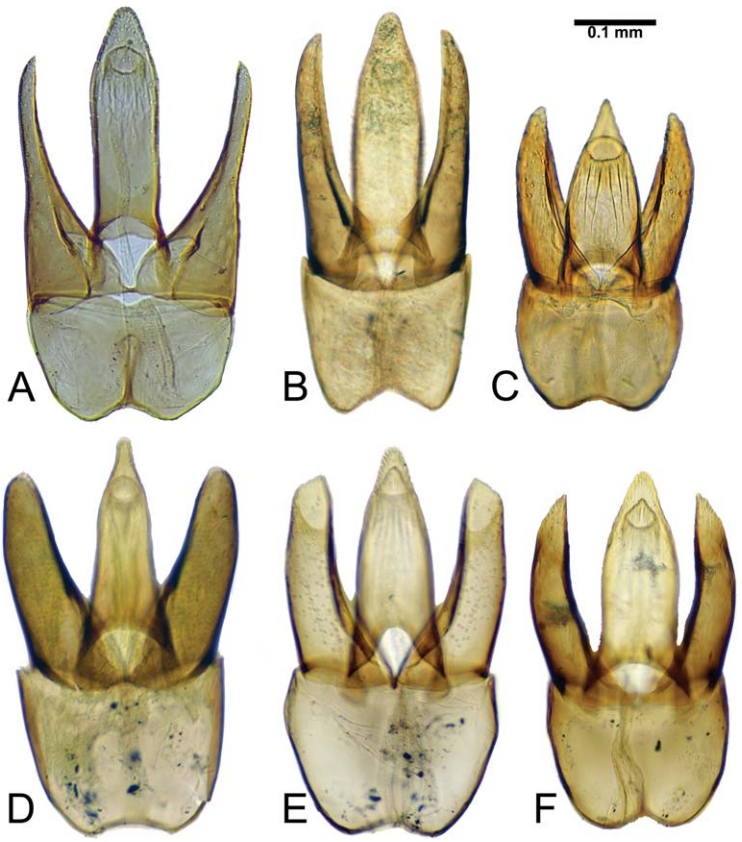

Fig. 4. Aedaegi of *Laricobius* species. (A) *L. osakensis* sp. nov. (B) *L. kangdingensis.* (C) *L. erichsonii.* (D) *L. laticollis.* (E) *L. rubidus.* (F) *L. nigrinus.* (Online figure in color.)

number is given in the listing). Information in {} is in Japanese.

Types are deposited at OMNH, unless otherwise indicated: HOLOTYPE—JAPAN: Kochi Prf, Yamada {Tosa-yamada}, 23-II-2007 *Tsuga* {backyard of building}, S. Shiyake leg. PARATYPES—JAPAN: Hyogo Prefecture, Kobe Municipal Arboretum, April 2008, Col. A. Lamb/ (two exs. 09-64.1 and 09-64.11, with slide mount of male and female genitalia, respectively. JPN: Osaka Prf, Takatsuki {Myo-on-ji, Nakahata, or Niryo}, 8-III, 16-III, 30-IV, 14-VII, 14-XI, 14-XII-2005, 1-V-2006 S. Shiyake or Y. Miyatake: *Tsuga sieboldii* (seven exs.). JPN: Hyogo Prf., Kobe Arboretum, 29-III-2005 or 24-IV-2006, S. Shiyake or Y. Miyatake (four exs.). JPN: Kyoto Prf, Kameoka {Uketa Shrine}, 16-III-2005 *Tsuga*, S. Shiyake leg (four exs.). JPN: Kyoto Prf., Kamigamo Exp. Sta., 16-II-2005 or 8-I-2008, S. Shiyake or A. Lamb & J. Chantos (two exs.). JAPAN: Nara Prf., Kamikitayama, Mt. Ohdai, 8-V-2006, ex. *Tsuga sieboldii*, Y. Miyatake (one ex.) JAPAN, Nara Prefecture, Nara Park, 34.696° N, 135.853° E, 27-IX-2009, leg S. Shiyake; ex. *Tsuga sieboldii;* (eight fully intact exs.; 1♂ and 1♀@: MCZ, NMNH, NZAC, OMNH). JAPAN, Nagano Prefecture, Matsumoto, Mt. Norikura, 36.12203° N, 137.58749° E, 2,030 m, 28-X-2009, A. Lamb & S. Shiyake; (one ex. 09-214.) JAPAN, Nagano Pref., Ootaki, Mt. Ontake, 35.86938° N, 137.51064° E, 2,050 m, 29-X-2009, A. Lamb & S. Shiyake (one exs. 09-215). JAPAN, Tochigi Prefecture, Nikko, Konsei Pass, 36.82° N, 139.39° E, 2,020 m, 1-XI-2009, A. Lamb & S. Shiyake (one ex. 09-216). JAPAN, Gunma Prefecture, Katashina, Mt. Nikko-Shirane, 36.81287° N, 139.34099° E, 1,550 m, 31-X-2009, Coll. A. Lamb & S. Shiyake (four exs. 09-219; two NZAC, two OMNH). JAPAN: Aomori Prf., Hirosaki, Hirosaki University, 15-V-2009, ex *Tsuga diversifolia*, S. Shiyake (one ex.). JPN: Kochi Prf, Hokigamine For. Pk., 6-I-2008, A. Lamb & J. Chantos (two exs.). JAPAN: Miyazaki Prf., Gokase, Mt. Mukosaka, 25-II-2010, ex *Tsuga sieboldii*, S. Shiyake (one ex.).

PARATYPES deposited at YPM with abdomens removed for extraction of DNA; male genitalia slide mounted and identically labeled: JAPAN, Kochi Prefecture, Tosa-Yamada, Hokigamine Forest Park, 6-I-2008, Coll.: S. Shiyake, A. Lamb; ex. *Tsuga sieboldii*, 33° 40.31′ N, 133° 41.97′ E (two exs. 08-26). JAPAN, Osaka Prefecture, XI-2006, Coll. A. Lamb (eight exs. 06-117). JAPAN, Osaka Prefecture, Takatsuki, Nakahata, 9-I-2008, Coll.: A. Lamb, S. Shiyake; ex. *Tsuga sieboldii;* 34° 57.68′ N, 135° 36.33′ E (one ex. 08-12). JAPAN, Osaka Prefecture, Takatsuki, Nakahata, Myo-on-ji Temple, 4-I-2008, Coll.: A. Lamb; ex. *Tsuga sieboldii*, 34° 57.57′ N, 135° 36.60′ E (two exs. 08-36.1, 08-36.2). JAPAN, Nara Prefecture, Nara Park, Wakakusa-Yama, 18-I-2008, Coll.: A. Lamb; ex. *Tsuga sieboldii;* 34° 41.467′ N,

Table 2. Measurements of head, prothorax, and elytra for six *Laricobius* species

Trait	*L. osakensis*	*L. kangdingensis*	*L. laticollis*	*L. nigrinus*	*L. rubidus*	*L. erichsonii*
HL	0.46 ± 0.05bc	0.43 ± 0.04ab	0.40 ± 0.05a	0.53 ± 0.05d	0.50 ± 0.02cd	0.48 ± 0.03bc
HW	0.60 ± 0.02c	0.54 ± 0.03a	0.57 ± 0.01ab	0.58 ± 0.02b	0.57 ± 0.01b	0.58 ± 0.03bc
HL/HW	0.77 ± 0.09ab	0.79 ± 0.04abc	0.71 ± 0.09a	0.93 ± 0.07d	0.87 ± 0.04cd	0.82 ± 0.04bc
PL	0.63 ± 0.04b	0.56 ± 0.03a	0.57 ± 0.02ab	0.61 ± 0.04bcd	0.59 ± 0.01abc	0.62 ± 0.04cd
PW	0.79 ± 0.05bc	0.72 ± 0.04a	0.82 ± 0.05bc	0.78 ± 0.02bc	0.78 ± 0.04b	0.83 ± 0.05bc
PL/PW	0.79 ± 0.04c	0.78 ± 0.02c	0.70 ± 0.02a	0.77 ± 0.03bc	0.76 ± 0.04bc	0.74 ± 0.02b
PAW	0.66 ± 0.02ab	0.63 ± 0.04a	0.68 ± 0.04c	0.63 ± 0.04a	0.67 ± 0.05ab	0.69 ± 0.04c
PPW	0.66 ± 0.07ab	0.60 ± 0.05a	0.68 ± 0.04b	0.67 ± 0.04b	0.65 ± 0.04ab	0.66 ± 0.05b
PAW/PPW	1.01 ± 0.08b	1.05 ± 0.05b	1.01 ± 0.03b	0.93 ± 0.03a	1.03 ± 0.02b	1.04 ± 0.05b
EL	1.87 ± 0.04bc	1.78 ± 0.07a	1.94 ± 0.05cd	1.99 ± 0.08d	1.84 ± 0.09ab	1.78 ± 0.02a
EW	1.22 ± 0.10a	1.16 ± 0.07a	1.14 ± 0.12a	1.22 ± 0.08a	1.14 ± 0.03a	1.18 ± 0.05a
EL/EW	1.54 ± 0.10ab	1.53 ± 0.08ab	1.71 ± 0.18c	1.63 ± 0.08bc	1.62 ± 0.05abc	1.51 ± 0.06a
EL/PL	3.00 ± 0.18ab	3.18 ± 0.13bc	3.40 ± 0.14d	3.28 ± 0.21cd	3.12 ± 0.14bc	2.90 ± 0.16a
Punct/mm	14.7 ± 1.0bc	15.8 ± 0.4cd	14.4 ± 1.7b	14.9 ± 0.9bc	15.4 ± 0.5bc	12.4 ± 0.9a
Scut cnt	7.67 ± 1.21b	7.00 ± 0.71b	6.90 ± 1.24b	7.83 ± 0.75b	7.20 ± 0.84b	5.40 ± 0.85a

Values are millimeters ± SD of the mean ($n = 10$). Values in a row followed by different letters are significantly different at the $P < 0.05$. Abbreviations for traits: L, length; W, width; H, head; P, pronotum; PAW, pronotum anterior width; PPW, pronotum posterior width; E, elytra (width of both); Punct/mm, number of punctures in the middle millimeter of the third row of elytral stria; and Scut cnt, number of punctures in the scutellary striola.

135° 51.152 E (two exs. 08-10). JAPAN, Hyogo Prefecture, Kobe, Kobe Arboretum, 9 or 14-I-2008 or IV 2008, Coll.: S. Shiyake, A. Lamb; ex. *Tsuga sieboldii*, 34° 44.45′ N, 135° 10.57′ E (15 exs. 08-09.1 to 08-09.3, 08-35.1 to 08-35.3, 09-64.2 to 09-64.10). JAPAN, Hyogo Prefecture, Kobe, Arima Onsen, 7-I-2008, Coll.: S. Shiyake, A. Lamb; ex. *Tsuga sieboldii*, 33° 47.73′ N, 135° 14.52′ E (two exs. 08-27). JAPAN, Kyoto Prefecture, Kyoto, Kamigamo Experimental Station, Kyoto University, 9-I-2008 or IV 2008, Coll.: A. Lamb, S. Shiyake; ex. *Tsuga sieboldii*, 35° 04.24′ N, 135° 45.83′ E (five exs. 08-28.1 to 08-28.2, 09-62.1 to 09-62.3). JAPAN, Nagano Prefecture, Mt. Norikura, 36.12224° N, 137.5810° E, 4 IV 2009 or 28-X-2009, Coll.: S. Shiyake, A. Lamb (four ex. 09-52, 09-213, 09-214). JAPAN, Nagano Prefecture, Shigakogen, Yamanouchi, 36.68213° N, 138.50032° E, V-2009, 17-X-2008, or 30-X-2009, Coll.: S. Shiyake, A. Lamb (four exs. 08-289, 09-51, 09-218). JAPAN, Nagano Pref., Ootaki, Mt. Ontake, 35.86938° N, 137.51064° E, 2,050 m, 29-X-2009, A. Lamb & S. Shiyake (one exs. 09-215) JAPAN, Tochigi Prefecture, Nikko, Konsei Pass, 36.79929° N, 139.42821° E, 3 IV or 1-XI-2009, Coll.: S. Shiyake; A. Lamb (two exs. 09-56 or 09-216.1). JAPAN, Tochigi Prefecture, Nikko, Nikko-Yumoto Spa, 36.803° N, 139.42° E, 31-XI-2009, Coll.: A. Lamb, S. Shiyake (four ex. 09-220.1). JAPAN, Gunma Prefecture, Katashina, Mt. Nikko-Shirane, 36.812° N, 139.341° E, 31-X-2009, Coll.: A. Lamb, S. Shiyake (10 ex. 09-119). JAPAN, Aomori Prefecture, Aomori, Mt. Ishikura, 40.637° N, 140.875° E, 20-X-2008; ex. *Tsuga diversifolia* (13 exs. 08-290.1 to 08-290.4, 09-57.1 to 09-57.4, 09-79.1 to 09-79.9).

Etymology. The specific name refers to Osaka Prefecture, where the species was first collected.

Distribution and Hosts. Widespread on the Japanese Islands of Honshu and Shikoku Islands with one specimen collected from Kyushu Island (Fig. 1). All specimens were collected from hemlock, *Tsuga sieboldii* Carr. and *Tsuga diversifolia* (Maxim.) Mast., infested with *A. tsugae.* Hemlock does not occur on Hokkaido Island, and we did not survey there. In the Kansai region, where monthly sampling was done, adults were collected every month except August but was most abundant from October to April. Larvae were collected between March and April. Both adults and larvae were observed to feed on *A. tsugae.* The beetle readily oviposits, and larvae were reared from egg to mature larva on this host. The larva enters the soil to pupate.

Results

Dimensional Measurements of Species. Relative measurements of the length and width of head, prothorax, and elytra are frequently used in the diagnosis of *Laricobius* species. In our comparative measurements of six *Laricobius* species, only a few of these measurements were useful in distinguishing species (Table 2). The relative length to width of the pronotum (PL/PW) is significantly less in *L. laticollis* than in other species, although the range of measurements has some overlap with *L. erichsonii.* The width of the pronotum at the anterior edge relative to the width at the posterior edge (PAW/PPW) is less in *L. nigrinus*, whereas in the other five species, the width across the anterior and posterior of the pronotum is subequal. The ratio of elytra length to pronotum length (EL/PL) overlaps for the three North American species, but may be useful to distinguish endemic species from the non-native species.

The density of punctures in the third row of the elytral striae of *L. erichsonii* is much less than for other species. The count of punctures in the scutellary striole is also less for *L. erichsonii* than the other species. The density of punctures was higher in *L. kangdingensis* that other species; in the other species density of punctures overlaps. Traditionally, a count is made of the total number of punctures in the third stria; however, counting the entire row is tedious due to the curvature of the apex of the elytra, and there is ambiguity in assigning punctures to a specific row, because rows may coalesce at the start and end of the

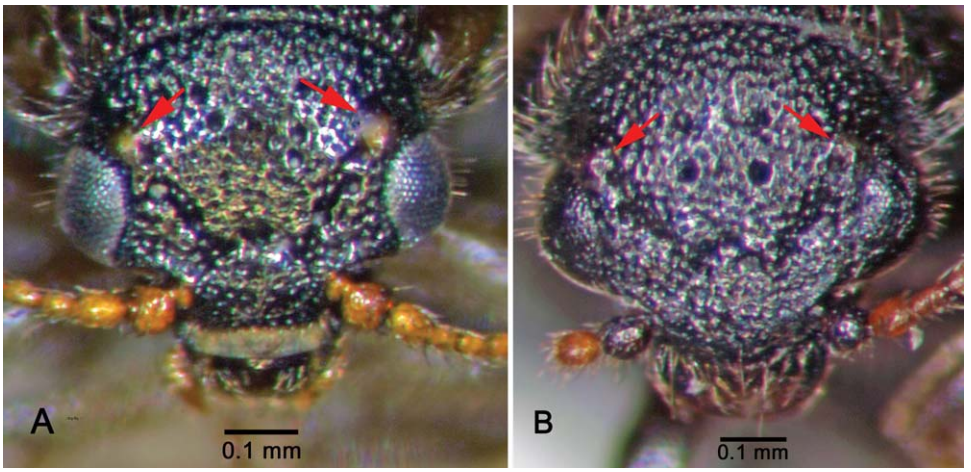

Fig. 5. Head showing pair of ocelli mesad of compound eyes, indicated by arrows. (A) *L. rubidus.* (B) *L. nigrinus.* (Online figure in color.)

rows. Punctures are most uniform and discernable in the midportion of the elytra; thus, we measured the middle, 1 mm—the portion covered by the eyepiece occular at 50×. An alternative is digital microscopy that provides a rapid method to precisely measure the diameter and distance between punctures within and between striae (Fig. 3E).

Partial Key to *Laricobius* Endemic and Imported to North America (* Indicates Non-Native Species)

1. Ocelli present (Fig. 5); aedeagus with thick median lobe, tapering from below midpoint to apex, parameres broad with blunt apex (Fig. 4C–F) 2
   Ocelli absent (Fig. 2B); aedeagus with slender median lobe, parameres slender with acute apex (Fig. 4A and B) 5
2. Elytra unicolorous; pronotum length to width <0.72, or transverse distance between anterior angles less than between posterior angles . . . 3
   Elytra usually bicolored; pronotum length to width >0.73 and transverse distance between anterior angles and between posterior angles subequal 4
3. Dorsal surfaces black or dark brown; pronotum with transverse between anterior angles narrower than across posterior angles. . . . . *L. nigrinus* Fender
   Pronotum and elytra light brown to tan with head darker; pronotum with transverse between anterior angles and between posterior angles subequal . . . . . *L. laticollis* Fall
4. Elytral punctures fine with dense, uneven spacing (>15/mm in middle of third row); ocelli small, <one fourth the length of eye . . . . . *L. rubidus* LeConte
   Elytral punctures larger and wider spaced (<13/mm in middle of third row); ocelli relatively large and oblong, about one fourth length of eye . . . . *L. erichsonii* Rosenhauer*
5. Frons with asymmetrical punctures, median vaulted, separated from clypeus by depression; pronotum with faint micropunctures (Fig. 6), posterior angle distinct with small tooth; phallobase of aedeagus without carina . . . . . *L. kangdingensis* Zilahi-Balogh & Jelínek*
   Frons relatively flat, with well-formed, round punctures, no frontoclypeal groove (Fig. 1B); pronotum with conspicuous micropunctures, posterior angle of pronotum without tooth; phallobase of aedeagus with median carina . . . . . *L. osakensis* sp. nov.*

Molecular Phylogeny. Partition homogeneity tests did not indicate conflict among gene regions ($P > 0.25$ for all comparisons). The combined data set was 2,732 nucleotides [COI: 657, EF1α: 1211 (1,014 without intron), *wingless:* 457, ITS2: 406]. The EF1α intron was

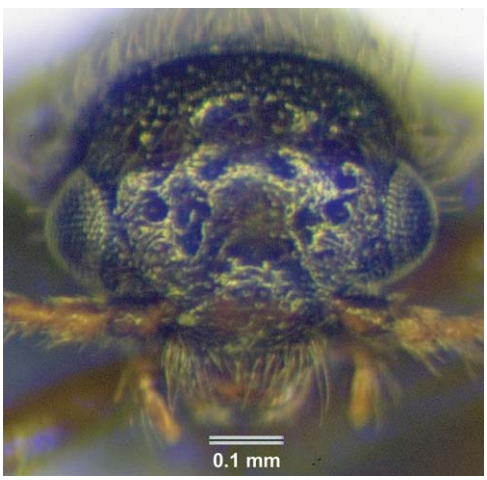

Fig. 6. Head of *L. kangdingensis* showing vaulted median of frons and depression separating frontoclypeal area. (Online figure in color.)

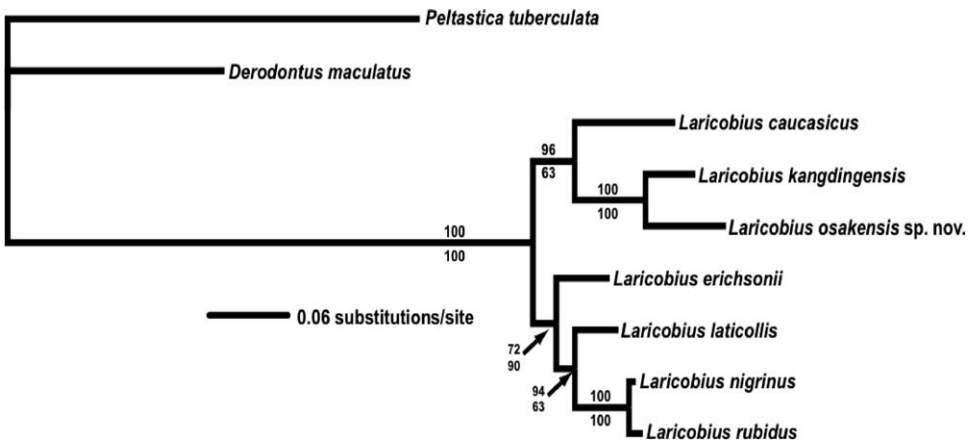

Fig. 7. Phylogram resulting from Bayesian analysis of COI, wingless, EF1α, and ITS2 sequence data showing relationships among *Laricobius* species present in North America with additional related species. Maximum parsimony analysis resulted in the same topology. Posterior probabilities are above and maximum parsimony bootstrap values are below branches.

not included because alignment was ambiguous, leaving 2,535 nucleotides for analysis. Amplification using primers EFS372 and Cho10Rev1 was not successful for *D. maculatus* resulting in a shorter EF1α sequence of 818 nucleotides for this sample. ITS2 sequences for the outgroups were not included because alignment with *Laricobius* sequences was ambiguous. The final data set contained 608 (24%) variable sites (COI: 197, EF1α: 218, *wingless:* 151, ITS2: 42), and 323 (13%) parsimony informative sites (COI: 133, EF1α: 90, *wingless:* 84, ITS2: 16). Maximum parsimony analysis resulted in a single most parsimonious tree with a length of 973 steps, consistency index of 0.776 and retention index of 0.610. Bayesian analysis used a mixed model of nucleotide substitution with separate, unlinked models for each partition (COI: GTR+I+G, EF1α: GTR+G, *wingless:* GTR+G, ITS2: K80+G), and resulted in an identical topology as the parsimony tree (Fig. 7).

The resulting tree contained two major clades: an Asian clade that includes *L. osakensis* from Japan, *L. kangdingensis* from western China, and *L. caucasicus* from the Caucasus Mountains, and a clade that includes the three North American species plus *L. erichsonii* from Europe, although the placement of *L. erichsonii* is not well supported. *L. nigrinus,* from western North America, is more closely related to the eastern species *L. rubidus* than it is to the other western species *L. laticollis.*

Molecular Diagnostics. Mean intraspecific K2P haplotype distance was 0.87% (range, 0.15–1.70%) for *L. osakensis,* 0.68% (range, 0.15–1.2%) for *L. nigrinus,* and 0.53% (range, 0.15–1.1%) for *L. rubidus.* Mean interspecific K2P distance was 15.2% (range, 13.8–15.8%) between *L. osakensis* and *L. nigrinus,* 15.3% (range, 14.3–16.2%) between *L. osakensis* and *L. rubidus,* and 2.2% (range, 1.4–2.8%) between *L. nigrinus* and *L. rubidus.* There were 60 nucleotide positions identified as "pure" diagnostics, i.e., the position contained one or more nucleotides that were unique to *L. osakensis* or to *L. nigrinus* + *L. rubidus* (Table 3). Of these, 49 contained a single nucleotide that is diagnostic for each group (e.g., position 15). Two sites had a single nucleotide unique to *L. osakensis* with multiple nucleotides unique to *L. nigrinus* + *L. rubidus* (e.g., position 351), nine had multiple nucleotides unique to *L. osakensis* with a single nucleotide unique to *L. nigrinus* + *L. rubidus* (e.g., position 273), and one diagnostic site (position 342) included all four nucleotides with a C or T in *L. osakensis* and an A or G in *L. nigrinus* + *L. rubidus.*

Discussion

L. osakensis can be distinguished from most species of *Laricobius* by the absence of ocelli. The only other described species, besides *L. osakensis,* lacking ocelli are *L. kangdingensis* found in southwestern China and *L. taiwanensis* found in Taiwan. These species differ in having more and deeper invaginations around the central area of the frons and between the antenna and frontoclypeal area. With respect to head punctation, *L. osakensis* resembles the North American species *L. rubidus* and *L. nigrinus* more than the Asian species.

Previously, a pair of small ocelli on the head mesad of the compound eyes was considered plesiomorphic for the Derodontidae (Lawrence and Hlavac 1979). Reports of actual observation of ocelli in *Laricobius* are the descriptions of *L. minutus* and *L. kovalevi* (Nikitsky and Lafer 1992) and Franz's (1958) detailed examination of *L. erichsonii.* We confirmed that ocelli are present in all known North American and European species as well as the Asian *L. baoxingensis* Zilahi-Balogh and Jelínek (2007), *L. bicolor* Háva (2008), *L. caucasicus* Rost (1893), *L. daliensis* Háva (2009c), *L. incognatus* Háva (2009d), *L. jizu* Háva (2010), *L. kovalevi* Nikitsky, *L. loebli* Jelínek and Háva (2001), *L. mirabilis* Háva and Jelínek (1999), *L. sahlbergi* Reitter (1883), *L. schawalleri* Háva and Jelínek (2000), and *L. wittmeri* (2009a). The three species without ocelli (*L. kangdingensis, L. osakensis,* and *L. taiwanensis*) occur on hemlock and feed on *A. tsugae.* The North Amer-

Table 3. Diagnostic characters to distinguish *L. osakensis* from *L. nigrinus* and *L. rubidus* from 657 bp of the mitochondrial COI gene

Nucleotide position	15	21	24	30	57[a]	60	66	105	111	117	196	198	201	205	211	222[a]	231
L. osakensis	T	A	T	T	A(1) C(20)	T	C(2) T(19)	C	C	T	C	T	T	C	T	T	T
L. nigrinus	C	C	C	A	T	A	G	T	T	C	T	A	A	T	C	T	A
L. rubidus	C	C	C	A	A	A	G	T	T	C	T	A	A	T	C	C	A

Nucleotide position	234	240	249	258	267	273[b]	286	318	324	327	333	336	339	342	351	375	384
L. osakensis	C	T	C	T	T	T	C	A	T	T	A	A	C	C(1) T(20)	A(20) C(1)	T	C
L. nigrinus	T	A	T	C	A	A(28)	T	C	A	A	T	T	T	A(26) G(2)	T	A	T
L. rubidus	T	A	T	C	A	A(1) C(1) G(56)	T	C(52) T(4)	A	A(55) G(1)	T	T	T	A(44) G(12)	T	A	T

Nucleotide position	400	405	411	414[b]	435	444	477	483	499	507	516	525	535	537	543	546	552
L. osakensis	C	T	C	T	C(19) T(2)	T	T	T	C	C	A	A	C	T	G	C	T
L. nigrinus	T	C	A(14) G(14)	A	A	C	A	A	T	A	T	T	T	A	A	T	A
L. rubidus	T	C	A(55) G(1)	A(5) G(51)	A	C	A	A	T	A(55) G(1)	T	T	T	A	A	T	A

Nucleotide position	555	561	577	579	580	588	606	618[b]	627	630	633	640	642	648	657[a]
L. osakensis	A	A	C	C(1) T(20)	C	C	C	T	A	T	C	T	A	G	T
L. nigrinus	T	C(1) T(27)	T	A	T	T	A	A	C(1) T(27)	A(27) G(1)	T	C	T	A	T
L. rubidus	T	C(1) T(55)	T	A	T	T	A	A(2) G(54)	T	A	T	C	T	A	C

Except where noted, all the included sites are pure diagnostics to distinguish *L. osakensis* from both *L. nigrinus* and *L. rubidus*. Results are based on sequences from 24 *L. osakensis* individuals (21 unique haplotypes), 114 *L. nigrinus* (28 haplotypes), and 213 *L. rubidus* (58 haplotypes). For polymorphic positions within species, the number of haplotypes possessing each nucleotide is shown in parentheses.

[a] Pure diagnostics for *L. nigrinus* or *L. rubidus*, and not *L. osakensis*.

[b] Pure diagnostics for *L. osakensis* and diagnostics with high confidence for *L. nigrinus* or *L. rubidus*.

ican *L. nigrinus* and *L. rubidus* both have small, rudimentary ocelli—the former species is usually collected from *A. tsugae* and occasionally from white pine adelgids in Western North American, whereas the latter species is usually collected from white pine adelgids and occasionally from *A. tsugae* in eastern North America.

Because of the planned release of *L. osakensis* in the eastern United States for the biological control of *A. tsugae*, it will be important to differentiate it from *L. rubidus*, which is endemic, and *L. nigrinus*, which has been introduced to the area. Positive identification in the field will be difficult because these three species can overlap in coloration. Furthermore, hybrids of *L. rubidus* and *L. nigrinus* have been confirmed previously (Havill et al. 2011). Although the latter two species generally have coloration that reflects their specific names, red and black, respectively, both species can be a dull, dark brown color. *L. osakensis* is also variable in coloration—some have striking bicolored reddish black elytra, resembling *L. rubidus*, and others are deep brown resembling *L. nigrinus.* However, dead specimens of the three species can be separated by microscopic examination. The absence (Fig. 2B) or presence of the ocelli (Fig. 5) is the surest character to separate *L. osakensis* from the North American species, although the ocelli may be difficult to see in *L. nigrinus* (Fig. 5B). The posterior angle of the pronotum is rounded without a projection or tooth for *L. osakensis*, whereas the posterial angle is more distinct and with a small projection in *L. nigrinus* and *L. rubidus.* The male genitalia of *L. osakensis* can be used to clearly separate it from the North American and European species. The latter have parameres that are much stouter in the apical half and a median lobe that begins to taper before the orifice (Fig. 4C–F), whereas *L. osakensis* has slender parameres and a median lobe that tapers after the orifice (Fig. 4A).

Only a few of the measurements of body parts were useful in distinguishing the six species (Table 2). The punctures in an elytral stria are fewer and coarser in the type species for the genus, *L. erichsonii*, than in other species examined. The diameter of the punctures depends on lighting (Figs. 2A and 3E) and is variable near the ends of the elytra; hence, this trait needs to be measured uniformly to be diagnostic. The relative length to width of the pronotum is unusually short in *L. laticollis* but similar in the other species examined. The pronotum of *L. nigrinus* is unusual in the transverse between the posterior angles being greater than the transverse between the anterior angles, whereas these distances are subequal or with the anterior transverse greater in the other five species. Lawrence and Hlavac (1979) and Zilahi-Balogh et al. (2006) also found the pronotum wider at the posterior edge in *L. nigrinus.* This characteristic was stable in material examined from California, Idaho, Washington, and Vancouver Island, BC, Canada; and it is especially useful in distinguishing *L. nigrinus* from *L. rubidus.*

The molecular information provides a sure method to identify not only *Laricobius* adults but also the larvae. *L. nigrinus* and *L. rubidus* were found to be very closely related sister species with few diagnostic nucleotide characters. Distinguishing these species using only COI sequence data is reliable, but tracking their genetic introgression will require highly polymorphic nuclear markers such as microsatellites. In contrast, distinguishing *L. osakensis* from *L. nigrinus* and *L. rubidus* with COI should be unambiguous. It is less likely (although possible) that *L. osakensis* will reproduce successfully with these species because it has been diverging longer and has considerable morphological differences such as the absence of ocelli and the shape of male genitalia. There are ample diagnostic sites to distinguish *L. osakensis* from the other species. These could be used to develop restriction fragment length polymorphism or real-time PCR assays that would save time and resources compared with sequencing.

The discovery, description, and genotyping of *L. osakensis* was motivated by the desire to obtain biological control of *A. tsugae* in the eastern United States. Although this article provides several tools to distinguish this species from other *Laricobius* species, native or previously introduced in North America, this does not obviate the need for biological control workers to retain vouchers of specimens recovered from the field after its release into new environments. There is considerable doubt about the establishment of *L. erichsonii* after the release of >130,000 adults in several localities in North America from 1951 to 1968 due to the lack of voucher specimens. It was considered established in both eastern and western Canada (Schooley et al. 1984). In the United States, recoveries were reported for only 1 or 2 yr after release; one report claiming recovery in 11 of 13 release sites and up to 1.6 km away, in the year after release (Amman and Speers 1965). Despite inquiries to all known possible repositories, we could verify through vouchers only one instance of *L. erichsonii* that were collected more than a year after release in North America. Harris and Dawson's (1979) report of recovery 15 yr after release is supported by seven specimens labeled N. Vancouver, 18-VII-1978 in the Canadian Forest Service collection, Victoria, BC, Canada (L. Humble, personal communication). A guide to the field identification of *L. erichsonii* published near the end of the release program (Brown and Clark 1962) indicated that *L. erichsonii* cannot be readily distinguished from *L. rubidus*, except for the shape of the male genitalia. The similarity of *L. rubidus* and *L. erichsonii* makes it plausible that recoveries of *L. erichsonii* in eastern North America may have been the native *L. rubidus.* In western North America, it is unlikely that adults of *L. erichsonii* were confused with the native *L. laticollis* and *L. nigrinus;* however, many of the reported recoveries were larvae, which are not identifiable to species. The morphological and DNA diagnostics described here, along with diligent vouchering of specimens, will allow accurate and detailed monitoring of postrelease establishment and impact of *Laricobius* species introduced as biological controls in eastern North America.

Acknowledgments

We thank the following people for loan of specimens: Gary Hevel (NMNH), Philip Perkins (MCZ), David Kavanaugh (California Academy of Sciences), Serge LaPlante (Canadian National Arthropod Collection) for sending specimens to Hamden, CT. (See Leschen 2011 for acknowledgment of specimens viewed by him in New Zealand or Europe.) We are grateful to Ashley Lamb and Yorio Miyatake for collecting additional specimens of *L. osakensis* and the following organizations for permission to collect insects in Japan: Kamigamo Experimental Station, Kyoto University; Kobe Municipal Arboretum; Nara Park Management Office; Hokigamine Forest Park, Kochi Prefecture; and Ministry of the Environment, Government of Japan. The molecular laboratory work by Joanne Klein and DeAdra Newman is greatly appreciated. We thank Alexi Sharov for translating Nikitsky and Lafer (1992) and Lee Humble Canadian Forest Service, Victoria, and Dan Quiring, University of New Brunswick, for checking collections. The work on which this article is based was partially supported by grants from USDA Forest Service Research to S.S. and from USDA Forest Service State & Private Forestry to R.A.B.L.

References Cited

Amman, G. D., and C. F. Speers. 1965. Release of predators of the balsam woolly aphid in North Carolina. USDA Forest Service, Southeastern Forest Experiment Station, Asheville, NC.

Brown, N. R., and R. C. Clark. 1962. Studies of predators of the balsam woolly aphid, *Adelges piceae* (Ratz.) (Homoptera: Adelgidae) X. Field identification of *Laricobius erichsonii* (Ratz) (Coleoptera, Derodontidae). Can. Entomol. 92: 237–240.

Cheah, C., M. E. Montgomery, S. Salom, B. L. Parker, S. Costa, and M. Skinner. 2004. Biological control of hemlock wooly adelgid, FHTET-2004-04. USDA Forest Service Forest Health Technology Enterprise Team, Morgantown, WV.

Clark, R. C., and N. R. Brown. 1960. Studies of predators of the balsam woolly aphid, *Adelges piceae* (Ratz.) (Homoptera: Adelgidae) VII. *Laricobius rubidus* Lec. (Coleoptera: Derodontidae), a predator of *Pineus strobi* (Hgt.) (Homoptera: Adelgidae). Can. Entomol. 92: 237–240.

Clark, R. C., D. O. Greenbank, D. G. Bryant, and J.W.E. Harris. 1971. *Adelges piceae* (Ratz.), balsam woolly aphid (Homoptera: Adelgidae), pp. 113–127. *In* Biological control programmes against insects and weeds in Canada 1959–1968. Commonwealth Agricultural Bureaux, Slough, United Kingdom.

Clausen, C. P. 1978. Chermidae, pp. 49–55. *In* C. P. Clausen (ed.), Introduced parasites and predators of arthropod pests and weeds: a world view. Agricultural Research Service, Washington, DC.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32: 1792–1797.

Franz, J. M. 1958. Studies on *Laricobius erichsonii* Rosenh. (Coleoptera: Derodontidae), a predator on chermesids. Part II External anatomy. Entomophaga 3: 165–196.

Ge, S.-Q., R. G. Beutel, and X.-K. Yang. 2007. Thoracic morphology of adults of Derodontidae and Nosodendridae and its phylogenetic implications (Coleoptera). Syst. Entomol. 32: 635–667.

Gatton, H. A., G.M.G. Zilahi-Balogh, S. M. Salom, and L. T. Kok. 2009. Biology and development of *Laricobius kangdingensis* Zilahi-Balogh and Jelínek, a newly discovered predator of *Adelges tsugae.* Biocontrol 54: 219–228.

Harris, J.W.E., and A. F. Dawson. 1979. Predator release program for balsam woolly aphid *Adelges piceae* (Homoptera: Adelgidae) in British Columbia, Canada, 1960–1969. J. Entomol. Soc. Br. Columbia 76: 21–26.

Háva, J. 2006. A world catalogue of the family Derodontidae (Coleoptera). Pol. Pis. Entomol. 75: 29–38.

Háva, J. 2008. *Laricobius bicolor* sp. n. (Coleoptera: Derodontidae: Laricobinae) from China. Calodema Suppl. Pap. 76: 1–4.

Háva, J. 2009a. Description of a new *Laricobius* species from China (Coleoptera: Derodontidae: Laricobinae) from China. Calodema 103: 1–4.

Háva, J. 2009b. Description of a new *Laricobius* species from Nepal (Coleoptera: Derodontidae: Laricobinae). Entomol. Basilensia Collections Frey 31: 115–118.

Háva, J. 2009c. *Laricobius daliensis* sp. n., a new beetle from China (Coleoptera: Derodontidae: Laricobinae) from China. Calodema 104: 1–3.

Háva, J. 2009d. New and interesting Derodontidae (Coleoptera) from Palaearctic region. Studies and Reports of District Museum Prague-East Taxonomical Series 5: 43–46.

Háva, J. 2010. *Laricobius jizu* sp. n., a new species from China (Coleoptera: Derodontidae: Laricobinae) with check-list of Chinese species. Calodema 112: 1–4.

Háva J., and J. Jelínek. 1999. A new species of the genus *Laricobius* (Coleoptera: Derodontidae) from China. Folia Heyrovskyana 7: 115–118.

Háva J., and J. Jelínek. 2000. *Laricobius schawalleri* sp. nov. from Nepal and the male of *Laricobius mirabilis* Háva and Jelínek, 1999, from China (Coleoptera: Derodontidae). Entomol. Z. 110: 184–185.

Havill, N. P., M. E. Montgomery, G. Yu, S. Shiyake, and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Ann. Entomol. Soc. Am. 9: 195–203.

Havill, N. P., R. G. Foottit, and C. D. von Dohlen. 2007. Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. Mol. Phylogenet. Evol. 44: 357–370.

Havill, N. P., G. Davis, J. Klein, A. Caccone, and S. Salom. 2011. Hemlock woolly adelgid biological control: Molecular methods to distinguish *Laricobius nigrinus, L. rubidus,* and their hybrids, pp. 25–28. *In* K. McManus and K. W. Gottschalk (eds.), Proceedings, 21st U.S. Department of Agriculture Interagency Research Forum on Gypsy Moth and Other Invasive Species. USDA Forest Service, Northeastern Research Station, Newton Square, PA. (in press).

Hebert, P.D.N., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator.* Proc. Natl. Acad. Sci. U.S.A. 101: 14812–14817.

Jelínek, J., and J. Háva. 2001. A new species of *Laricobius* (Coleoptera: Derodontidae). Rev. Suisse Zool. 108: 149–152.

Lawrence, J. F., and T. F. Hlavac. 1979. Review of the Derodontidae (Coleoptera: Polyphaga) with new species from North America and Chile. Coleopt. Bull. 33: 369–414.

Lawrence, J. F., R. G. Beutel, R.A.B. Leschen, and S. A. Ślipiński. 2010. Chapter 2. Glossary of morphological terms, pp. 9–20. *In* R. A. Leschen, B., R. G. Beutel, and J. F.

Lawrence (eds.), Handbook of zoology, vol. 2. Walter de Gruyter, Berlin, Germany.

Leschen, R.A.B. 2011. World review of *Laricobius* (Coleoptera: Derodontidae). Zootaxa (in press).

Leschen, R.A.B., and R. G Beutel. 2010. Chapter 5.1. Derodontidae, pp. 180–185. *In* R.A.B. Leschen, R. G. Beutel, and J. F. Lawrence (eds.), Handbook of zoology, vol. 2. Walter de Gruyter, Berlin, Germany.

Maddison, W. P., and D. R. Maddison. 2005. MacClade 4: analysis of phylogeny and character evolution. Sinauer, Sunderland, MA.

Mausel, D. L., S. M. Salom, L. T. Kok, and G. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. Environ. Entomol. 39: 440–448.

McKenna, D. D., A. S. Sequeira, A. E. Marvaldi, and B. D. Farrell. 2009. Temporal lags and overlap in the diversification of weevils and flowering plant. Proc. Natl. Acad. Sci. U.S.A. 106: 7083–7088.

Montgomery, M. E., and S. M. Lyon. 1996. Natural enemies of adelgids in North America: their prospect for biological control of *Adelges tsugae* (Homoptera: Adelgidae), pp. 89–102. *In* S. M. Salom, T. C. Tigner, and R. C. Reardon (eds.), Proceedings of the First Hemlock Woolly Adelgid Review. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Nikitsky, N., and G. S. Lafer. 1992. 47. Fam. Derodontidae, pp. 40–44. *In* P. A. Ler, (ed.), Key to the identification of insects of the Far East of the USSR 3: 704. Nauka, St. Petersburg Nauka, Russia.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Reitter, E. 1883. Neue Coleopteren aus Russland und Bermkungen uber bekannte Arten. Rev. Mensuelle Entomol. 1: 40–44.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Rost, C. 1893. Neue oder wenig bekannte caucasische Coleopteren. Entomol. Nachrichten 19: 338–344.

Sarkar, I. N., P. J. Planet, and R. DeSalle. 2008. CAOS software for use in character-based DNA barcoding. Mol. Ecol. Resour. 8: 1256–1259.

Schooley, H. O., J.W.E. Harris, and B. Pendrel. 1984. *Adelges piceae* (Ratz.), balsam woolly adelgid (Homoptera: Adelgidae), pp. 229–234. *In* J. S. Kelleher and M. A. Hulme (eds.), Biological control programmes against insects and weeds in Canada 1969–1980. Commonwealth Agricultural Bureax, Farnham Royal, Slough, England.

Swofford, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, MA.

Wild, A. L., and D. R. Maddison. 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. Mol. Phylogenet. Evol. 48: 877–891.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315–322. *In* M. Innis, D. Gelfand, J. Sninsky, and T. White (eds.), PCR protocols: a guide to methods and applications. Academic, San Diego, CA.

Yu, G., and M. E. Montgomery. 2007. A new species of *Laricobius* (Coleoptera: Derodontidae) from Taiwan. Formosan Entomol. 27: 341–347.

Zilahi-Balogh, G.M.G., L. M. Humble, L. T. Kok, and S. M. Salom. 2006. Morphology of *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of the hemlock woolly adelgid. Can. Entomol. 138: 595–601.

Zilahi-Balogh, G.M.G., J. Jelínek, T. J. McAvoy, S. M. Salom, and L. T. Kok. 2007. Two new species of *Laricobius* (Coleoptera: Derodontidae) from China, and a key to *Laricobius* in the southeastern Palaearctic. Proc. Entomol. Soc. Wash. 109: 377–384.

Received 8 September 2010; accepted 12 January 2011.