Animal Cells and Systems
Vol. 16, No. 2, April 2012, 154–161

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A review of the genus *Adoxophyes* (Lepidoptera Tortricidae) in Korea, with description of *A. paraorana* sp. nov.

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(*Received 23 January 2011; received in revised form 2 April 2011; accepted 6 April 2011*)

We reviewed the genus *Adoxophyes* (Lepidoptera: Tortricidae) from Korea and recognized three species of the genus. Among them, *A. paraorana* sp. nov., formerly misidentified as *A. orana* in Korea, is described as new to science. *Adoxophyes orana*, a notorious pest known in most Eurasian countries for a long time, turns out to be only recently introduced or found in Korea. Photographs of the adults and genitalia of the species are provided. Specific distinction was supported by the COI barcode study.

Keywords: Tortricidae; *Adoxophyes*; *orana* complex; *A. paraorana*; Korea

Introduction

The Tortricidae comprises more than 9000 species in the world (Brown 2005). The larvae are known as typical leaf-rollers. Among them, Tortricinae includes several of the most notorious species of economic importance (Van der Geest and Evinhuis 1991). Nevertheless, many genera and species complexes within the subfamily remain taxonomically unresolved. One of the species complexes in East Asia is the *orana* species complex (Park et al. 2008).

Adoxophyes orana (Fischer von Rőslerstamm, 1834) was previously classified as *Tortrix orana* in Europe in the 1800s and the current name was adopted in 1952 (Park et al. 2008). In Japan, four species were recorded with description of two new species, *A. honmai* and *A. dubia*, which had been confused in identification. The nominal history of *Adoxophyes* species was recently summarized (Park et al. 2008).

In Korea, only one species, *A. orana,* had been known until recently (Byun et al. 1998). However, since another species, though its specific name was unconfirmed, other than *A. orana* was first recognized by Lee et al. (2005) and Yang et al. (2005), the strong possibility of the existence of two additional species, *A. honmai* and a possibly new *Adoxophyes* species, in Korea was suggested based on differences of COI barcode sequences and differential pheromone responses (Park et al. 2008; Yang et al. 2009). However, because of the morphological similarities among the species, a detailed morphological comparison of the related species has been needed.

In this study, we recently examined all the available material collected from field trips and pheromone traps in South Korea. Morphological comparison was done for the three species recognized here. To corroborate the morphological identification, we also compared the COI sequences of multiple samples from each species. Among the three species, *A. paraorana* Byun, sp. nov., long misidentified as *A. orana*, is described as new to science. *Adoxophyes honmai* Yasuda is morphotaxonomically reported in Korea for the first time. *Adoxophyes orana*, only recently collected from the Cheonan area in Korea, is taxonomically reported as new to Korea.

Materials and methods

The specimens for the morphological and molecular study were from the following institutions: the Korea National Arboretum, Pocheon (KNA), the Natural History Museum, Hannam University, Daejeon (HUNHM), National Institute of Horticultural and Herbal Science, Suwon (NIHHS), Chungbuk National University Entomological Collection, Cheongju (CBNE), and National Plant Quarantine Service (NPQS).

Morphological study

The available genitalia for the species were prepared on slide glass with Euparal mountant, and the illustrations for each species were taken by digital camera, AxioCam MRc 5 (Carl Zeiss, Germany) attached to a

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ISSN 1976-8354 print/ISSN 2151-2485 online

http://dx.doi.org/10.1080/19768354.2011.611175
http://www.tandfonline.com

microscope, Axio Imager A1 (Carl Zeiss, Germany). The color standard for the redescription of adults was based on the Methuen Handbook of Colour (Kornerup and Wanscher 1978). Taxonomic assignment of each species in this study was following Brown (2005).

Taxon sampling for DNA analysis

DNA sequence data were obtained from the species collected from various regions (Table 1) and superficially recognized as a species in *orana* complex by morphology. *Adoxophyes orana* has recently been collected only from Cheonan. Since *A. orana* was originally reported from Europe, we included a specimen from Norway. In addition, one sample of *A. honmai* from Japan was included as the species was also reported for the first time from Japan. For an outgroup, *Grapholita molesta*, a species of a closely related genus to *Adoxophyes*, was included.

Molecular study

Total genomic DNA was extracted from dried specimens using a DNeasy Tissue Kit (Qiagen, Germany), and 20–50 μl of the extract was stored in a freezer (−20°C) until being used for PCR amplification. Legs or thorax were used for DNA extraction. The rest of the body parts were saved for further DNA analysis or for vouchering. PCR was carried using AccuPower® PCR PreMix (Bioneer, Korea) under the following conditions: an initial denaturation step at 94°C for 7 min; 35 cycles at 94°C for 1 min, annealing temperatures (50–57°C) for 1 min, extension at 72°C for 2 min; and final extension for 7 min at 72°C, or amplified with Ex-Taq (TaKaRa, Japan) under the following conditions: an initial denaturation step at 95°C for 2 min; 40 cycles at 95°C for 10 sec, annealing temperatures (55°C) for 30 sec, extension at 72°C for 45 sec; and final extension for 5 min at 72°C. The primer pair, LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) was used to amplify a 658 bp fragment of the COI gene (Folmer et al. 1994). To confirm the success of DNA amplification, electrophoresis was conducted using 1x TAE buffer on 1% agarose gel. The PCR product was then purified with a PCR purification kit (Bioneer, Korea, or Intron, Korea) and sent to a commercial company (YeBT, Seoul, Genotech, Daejeon, or Solgent, Daejeon) for sequencing. Pairwise genetic distances based on both Kimura 2-parameter (K2P) model and p-distance were calculated using MEGA 5 program (Kumar et al. 2008). Neighbor-joining (NJ) analysis with maximum composite likelihood option was performed in MEGA, too. Bootstrap values were estimated with 1000 replicates.

Results and discussion

Systematic accounts

Adoxophyes Meyrick, 1881

Table 1. List of the sequenced samples with code number, GenBank accession number and collection locality.

Species	Code	GenBank Acc. #	Locality
Grapholita molesta	GM-1	JF733841	GB: Bonghwa-gun
Adoxophyes honmai	H-1	JF733842	JN: Naju-si
	H-49	JF733843	JJ: Jeju-si
	H-168	JF733844	JJ: Jeju-si
	H-J	JF733845	Japan
Adoxophyes orana	O-143	JF733848	CN: Cheonan-si
	O-144	JF733849	CN: Cheonan-si
	O-145	JF733850	CN: Cheonan-si
	O-1	JF733846	CN: Cheonan-si
	O-2	JF733847	CN: Cheonan-si
	O-N	JF733851	Norway
Adoxophyes paraorana	P-50	JF733838	GG: Suwon-si
	P-51	JF733839	GG: Suwon-si
	P-55	JF733840	GB: Yecheon-gun
	P-32	JF733833	CB: Chungju-si
	P-33	JF733834	CB: Chungju-si
	P-34	JF733835	CB: Chungju-si
	P-40	JF733836	CB: Chungju-si
	P-41	JF733837	CB: Chungju-si

Abbreviations for provinces are as follows: CB, Chungbuk; CN, Chungnam; GB, Gyeongbuk; GG, Gyeonggi; JJ, Jeju; JN, Jeonnam.

Adoxophyes Meyrick, 1881, Proc. Linn. Soc. N.S.W. 6: 429. Type species: *Adoxophyes heteroidana* Meyrick, 1881 (E. Australia) by monotypy

Adoxophyes paraorana Byun[1] (Figures 1, 4, 7, 10, 13). Types. Holotype. ♂, Namyangju, GG, 11.VI.2008, (C.Y. Yang)-gen. slide. no. 789-coll. KNA. Paratypes. 3♂, 2♀, Namyangju, GG, 11.VI.2008, (C.Y. Yang)-gen. slide. no. 789-coll. KNA; 2♂, 2♀, Namyangju, GG, 11.VI.2008, (C.Y. Yang); 1♂, Cheongju, CB, 13.X.1997 (C.H. Park)-gen. slide no. 346-coll. HUNHM.

Diagnosis. This species is very similar to *A. orana* (Fischer von Rőslerstamm) externally, but it can be distinguished by the shape of transtilla in male genitalia with a medially disconnected part forming a golf club-like, slightly curved upward with numerous short spines dorso-terminally.

Adults. Male (Figure 1). Wingspan, 14–18 mm. Head, palpus and thorax brownish-ochreous. Abdomen grayish ochreous with large, pale, fuscous tufts. Forewing elongate, gently curved medially, with a broad costal fold reaching just before middle of costa; apex not acute; termen slightly oblique, with round tornus. Ground color of forewing brownish ochreous yellow, rather stronger and deeper than those of the other species, *A. honmai* and *A. orana*; markings reddish-brown, narrowly edged with pale yellowish color along inner and outer margins of each fascia; basal patch originated from near 1/3 of costa to 1/3 of dorsum, with an additional fascia, blackish brown, originated from right after base of dorsum to the end of basal fascia; median fascia from near middle of costa to 2/3 of dorsum, narrowly edged with pale ochreous, moderately broad along upper half, then branched into two fasciae near middle of forewing; preapical fascia running from near 2/3 of costa to tornus, broad with narrow edge along inner and outer margin. Cilia brownish yellow, slightly suffused with grayish brown. Hindwing light ochreous-yellow, a bit darker posteriorly. Female (Figure 4). Wingspan 16–18 mm. Head, palpus and thorax brownish yellow suffused with grayish brown. Abdomen grayish ochreous, suffused with brownish yellow. Forewing gently arched, rather straight posteriorly, apex somewhat rounded, termen slightly oblique with obtuse tornus. Ground color reddish brown suffused with grayish brown, rather dull; basal patch originated from near 1/3 of costa to near 1/3 of dorsum, rather weak in coloration, with a small conspicuous erect triangular blackish brown patch near 1/3 of dorsum; median fascia from near middle of 2/3 of dorsum, moderately broad along upper half, then branched near middle of forewing; preapical fascia a bit broad, running from near 4/5 of costa to tornus, slightly concaved at around 1/3 posteriorly, markings rather reduced in female. Cilia brighter than those in male. Hindwing somewhat yellowish.

Male genitalia (Figures 7, 10). Uncus moderate, spatulate, rounded terminally, slightly narrowed medially. Gnathos arms somewhat broad, upcurved apically, rounded terminally. Valva simple with a short, triangular brachiola. Transtilla lobes moderately broad, beaked, touching medially, with numerous spines of variable sizes dorso-terminally. Juxta heart symbol-shaped, slightly concaved medially, rather broad. Aedeagus cylindrical, slightly curved inwardly at around 2/5, with several short, slender cornuti in vesica.

Female genitalia (Figure 13). Ovipositor lobe large. Ostium opening small, rounded. Antrum lightly sclerotized. Inception of ductus seminalis lateral at anterior edge of antrum. Bulla seminalis slightly smaller than corpus bursae. Ductus bursae membranous except for granular area anterior to antrum. Corpus bursae spherical, one signum near junction of ductus bursae and corpus bursae, hook-shaped, nearly circular, strongly sclerotized at base.

Distribution. Korea

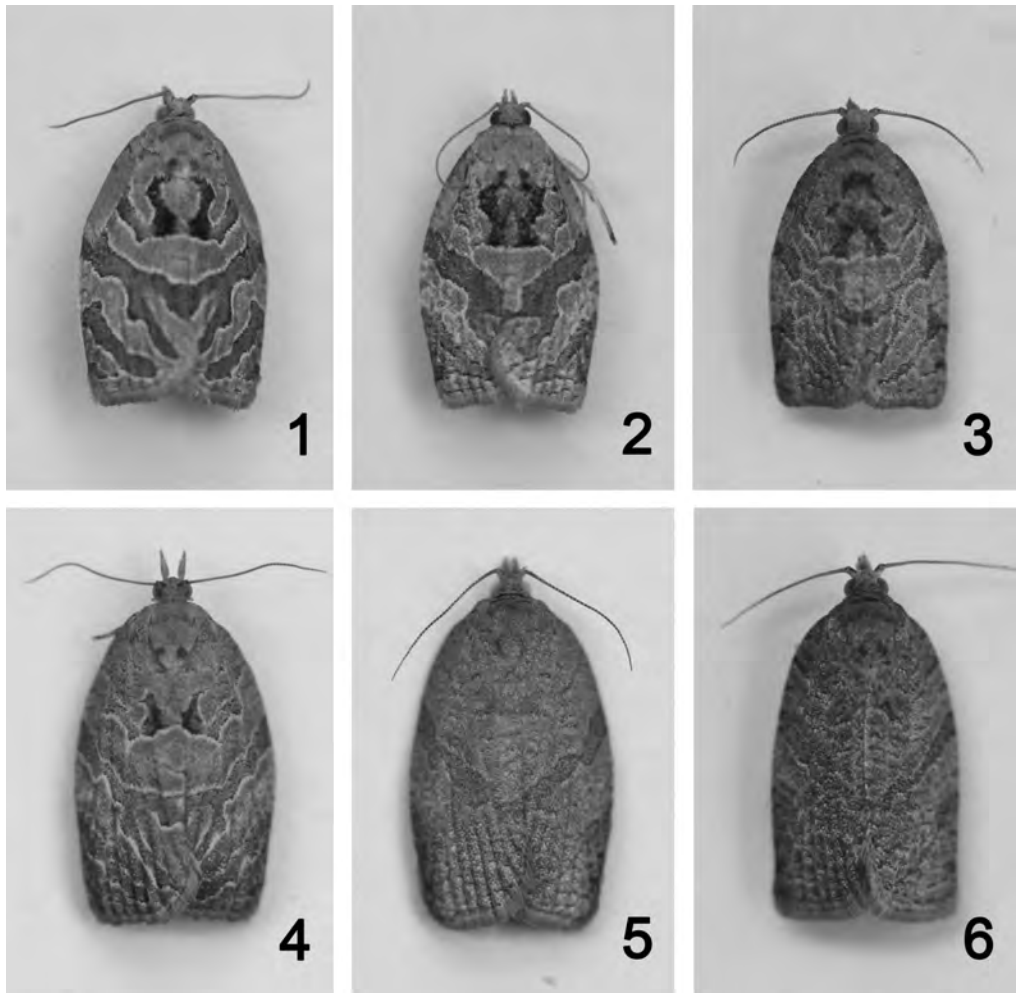
Etymology. The specific name of the new species is derived from *A. orana* (Fischer von Rőslerstamm), which means a closely allied species to *A. orana*.

Remarks. This species has been confused with *A. orana* in Korea for many years. The possibility of establishing a new species has been suggested based on COI and 18S rDNA comparison (Park et al. 2008).

Adoxophyes honmai Yasuda[2] (Figures 2, 5, 8, 11, 14)
Adoxophyes honmai Yasuda, 1998, Trans. Lepid. Soc. Japan 49(3): 164.

Diagnosis. This species is very similar to *A. orana* (Fischer von Rőslerstamm) externally, but it can be distinguished by the shape of transtilla in male genitalia, which is completely disconnected at middle.

Adults. Male (Figure 2). Wingspan 14 mm. Forewing elongate, slightly curved near middle, with broad costal fold; apex obtuse; termen slightly oblique, rounded around tornus. Ground color grayish brown, suffused with reddish brown, darker than that in *A. paraorana* sp. nov.; markings grayish brown, with narrow edge along margins of fasciae; basal patch characteristic with a semicircular spot, distinct, blackish brown, covering near anterior 1/3 of dorsum; median fascia from before middle of costa, broader in upper half, then branched into two fairly narrow fasciae towards 4/5 of dorsum and tornus, respectively; costal patch narrow running from near 4/5 of costa to near 1/3 of termen towards tornus. Cilia ochreous-yellowish, slightly mixed with grayish brown. Hindwing ochreous-yellowish, a bit darker posteriorly. Female



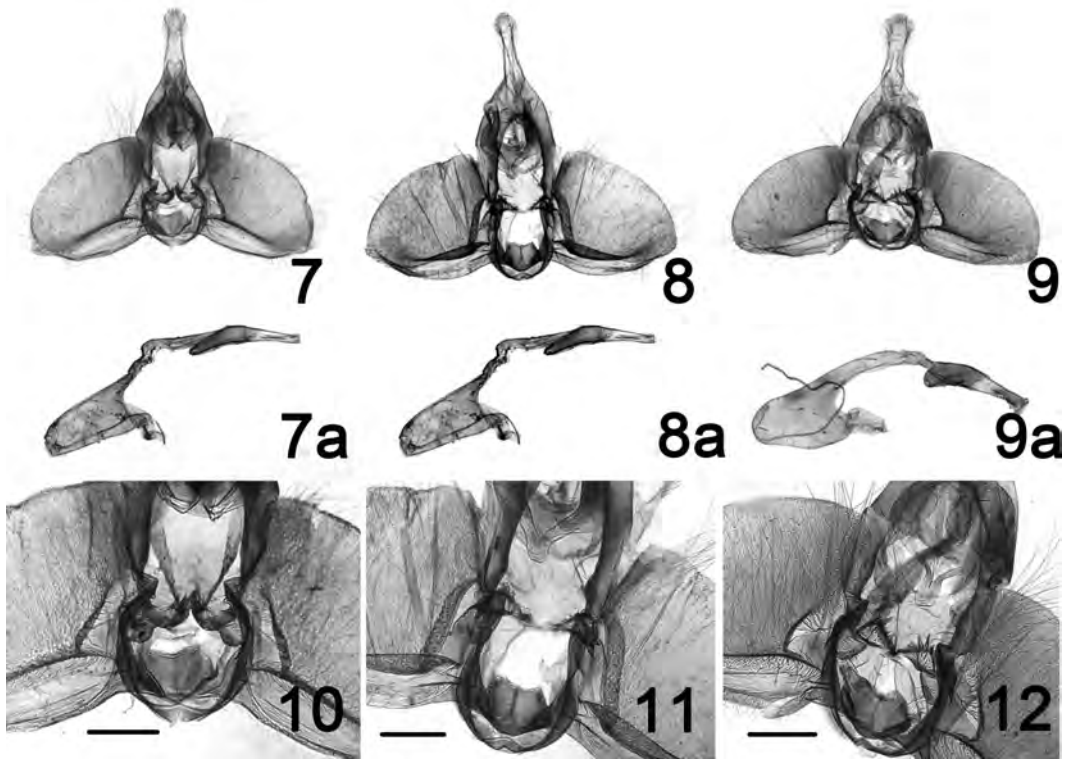
Figures 1–6. Adults.

(Figure 5). Wingspan 16–18 mm. Forewing with costa rather straight posteriorly, with obtuse apex, termen slightly oblique, tornus a little bit rounded; basal patch with apparent small erect triangular grayish-brown patch near 1/3 of dorsum, rather weaker than that in *A. paraorana* sp. nov. in coloration; median fascia from near middle of costa to near 2/3 of dorsum, a bit narrow and not branched at basal half; preapical fascia running from near 4/5 of costa to tornus, rather weak in coloration at distal half. Cilia ochreous-yellowish, brighter than in male. Hindwing ochreous yellow, somewhat brighter than in male. Cilia light ochreous yellow.

Male genitalia (Figures 8, 11). Uncus with smaller top than in *A. paraorana* sp. nov., apical membranous part rather small, middle part of uncus narrower than that of *A. paraorana* sp. nov. Gnathos arms a bit strong and broad, upcurved apically, rounded terminally. Valva simple with one nail-shaped brachiola. Transtilla lobes moderately broad, beaked, not touching each other, numerous strong spines of different sizes dorsally. Juxta heart-shaped, slightly concaved medially, rather broad. Aedeagus cylindrical, slightly curved inwardly near 2/5, several short, slender cornuti in vesica.

Female genitalia (Figure 14). Very similar to those of *orana* but with much smaller corpus bursae and with distinctly longer ductus bursae.

Material examined. 1♂, Cheongju, CB, 15.V.1997 (C.H. Park); 4♂, 5♀, same locality, 14.VII.1997 (C.H. Park): 1♀, same locality, 27.V.1998 (J.S. Chae): 1♂, Naju, JN, 25.VII. 1996 (S.K. Jang): 8♂, 3♀, same locality, 30.VII.2008 (C.Y. Yang)-gen. slide no. 791, 792; 1♂, same locality, 5.IX. 1996 (S.K. Jang): 5♂, 5♀, Gwangju, JN, 15.VII.1999 (J.M. Lee and K.S. Han): 2♂, Suncheon, JN, 1.VII.1992 (S.S. Kim); 1♀, Mt. Halla, JJ, 25.V.1988 (K.T. Park) gen. slide no. 2575: 1♂, Mokseokwon, JJ, 29.V.1992 (B.K. Byun)-gen. slide no. 351: 1♂, Jeju-si, JJ, 22.IV.1999 (B.K. Byun): 1♀, Jeju-si, JJ, 8.VII.1996 (Y.G. Kim)-gen. slide. no. 375; 1♀, same locality, 26.IX.1996 (Y.G. Kim) -coll. HUNHM.



Figures 7–12. Male genitalia.

Distribution. Korea and Japan

Remarks. In Japan, this species is widely distributed in southern Honshu, where it is an important pest, damaging to tea (Yasuda 1998). Existence of this species in Korea has been reported with both molecular and pheromonal evidence (Lee et al. 2005, 2006; Yang et al. 2005; Park et al. 2008).

Adoxophyes orana (Fischer von Rőslerstamm)[3] (Figures 3, 6, 9, 12, 15)

Adoxophyes orana Fischer von Rőslerstamm, 1834 (*Tortrix*), Abbild. Berich. Ergänz. Schmett. –Kunde 1: 13. TL: Germany (Bohemian Hungary). Syntype: Unknown.

Adoxophyes fasciata Walsingham, 1900, Ann. Mag. nat. Hist. (7)5: 482; Issiki, 1922: 283.

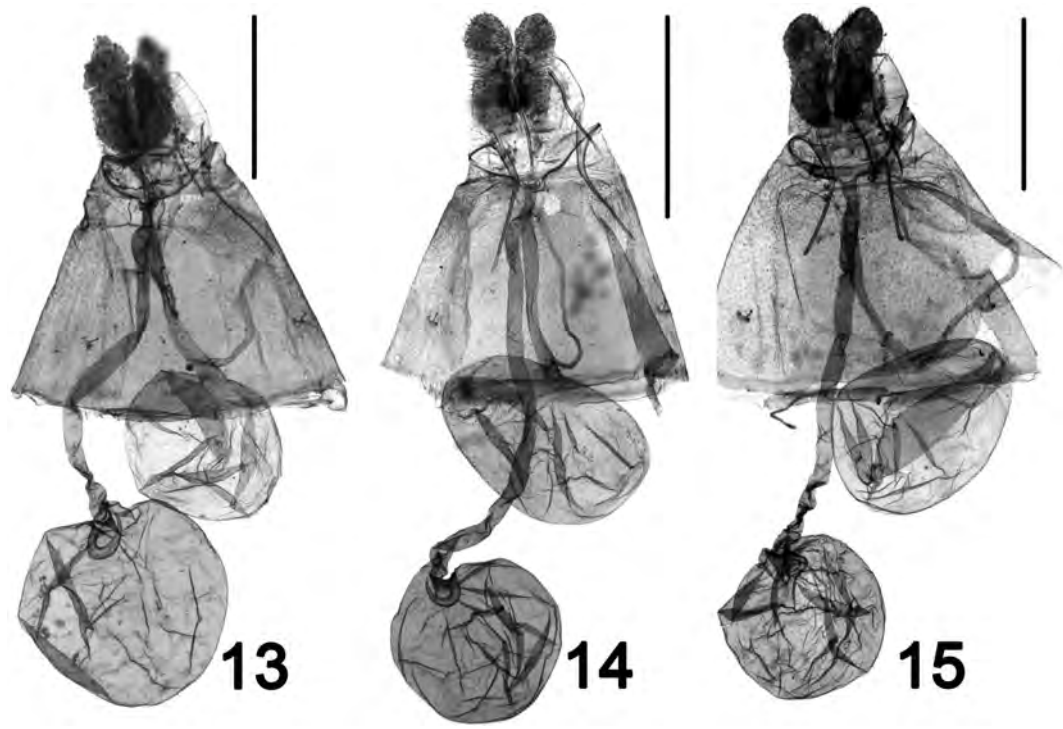
Adoxophyes orana fasciata: Yasuda, 1975: 129; Kawabe, 1982, I: 72, II: 161, pl. 16: 37–38; Shirasaki and Yamada, 1983: 33; Sugie et al., 1984: 156.

Adoxophyes orana: Yasuda, 1956: 23, 24; Honma, 1965: 35; Honma, 1970: 47; Byun et al., 1998: 12

Diagnosis. This species is very similar to *A. paraorana* sp. nov. externally, but it can be distinguished by the shape of transtilla in male genitalia, which is connected medially and with numerous short spines dorsally, and by juxta, which is slightly curved downwardly at middle.

Adults. Male (Figure 3). Wingspan 14–18 mm. Forewing elongate, slightly curved near middle, with a broad costal fold; apex obtuse; termen slightly oblique, rounded around tornus. Ground color and patterns similar to *A. paraorana* sp. nov., but rather weaker in coloration than the latter, also with a rather indistinct semicircular spot of bascal patch; medial and preapical fasciae somewhat narrower than the allied species. Female (Figure 6). Wingspan 18–28 mm. Forewing with costa rather straight posteriorly, with obtuse apex, termen slightly oblique, tornus a bit rounded; ground color and pattern similar to those of *A. paraorana* sp. nov. and *A. honmai*, rather weaker in coloration than that of *A. paraorana* sp. nov., but slightly stronger than that of *A. honmai*. Hindwing ochreous yellow, somewhat brighter than that of male. Cilia light ochreous yellow.

Male genitalia (Figures 9, 12). Uncus with broader top than in *A. paraorana* sp. nov., rounded terminally, middle part of uncus broader than in *A. paraorana* sp. nov. Gnathos arms a bit strong and broad, upcurved apically, rounded terminally, a bit longer than in *A. paraorana* sp. nov. Valva simple with a nail-shaped brachiola. Transtilla lobes moderately broad, beaked, touching each other, numerous strong spines of different size dorsally. Juxta heart symbol-shaped, slightly



Figures 13–15. Female genitalia.

concaved medially, rather broad. Aedeagus cylindrical, somewhat broader than in *A. paraorana* sp. nov., with several short cornuti in vesica.

Female genitalia (Figure 15). Ovipositor lobe large. Ostium opening small, rounded. Antrum lightly sclerotized. Inception of ductus seminalis lateral at anterior edge of antrum. Bulla seminalis slightly smaller than corpus bursae. Ductus bursae mebranous except for the granular area anterior to antrum. Corpus bursae spherical, signum hook-shaped, forming a strongly sclerotized base at junction of ductus bursae and corpus bursae.

Material examined. 7♂, Hongneung, Seoul, 2.VI.1996 (B.K. Byun); 1♂, same locality, 8.VI.1996 (B.K. Byun); 1♂, same locality, 11.VII.1996 (B.K. Byun); 1♂, same locality, 16.VIII.1996 (B.K. Byun); 1♂, 1♀, same locality, 21.VIII.1996 (B.K. Byun); 1♂, same locality, 26.VIII.1996 (B.K. Byun); 1♂, same locality, 29. VIII.1996 (B.K. Byun); 2♂, 2♀, same locality, 5.IX. 1996 (B.K. Byun); 1♂, 1♀, same locality, 6.X.1996 (B.K. Byun); 1♂, 3♀, same locality, 9.IX.1996 (B.K. Byun); 3♂, same locality, 10.IX.1996 (B.K. Byun); 2♂, 2♀, same locality, 11.IX.1996 (B.K. Byun); 1♀, same locality, 12.IX.1996 (B.K. Byun); 2♂, 3♀, same locality, 12.IX.1996 (B.K. Byun); 1♂, same locality, 13.X.1996 (B.K. Byun); 1♂, same locality, 14.X.1996 (B.K. Byun); 2♀, same locality, 16.IX.1996 (B.K. Byun); 1♂, 2♀, same locality, 23.IX.1996 (B.K. Byun); 2♂, same locality, 28.IX.1996 (B.K. Byun); 1♀, same locality, 29.IX.1996 (B.K. Byun); 1♀, Hongneung, Seoul,

Table 2. P-distance (top right) and K2P-distance (bottom left) matrix for some representative samples. P-32, P-33 and P-50: *Adoxophyes paraorana*; H-J and H-49: *A. honmai* (H-J is a Japanese specimen); O-N and O-143: *A. orana* (O-N is a Norwegian specimen); and GM-1 is *Grapholita molesta* as an outgroup.

	P-32	P-33	P-50	H-J	H-49	O-N	O-143	GM-1
P-32		0.003	0.006	0.048	0.048	0.060	0.060	0.114
P-33	0.003		0.003	0.044	0.044	0.057	0.057	0.111
P-50	0.006	0.003		0.044	0.044	0.057	0.057	0.111
H-J	0.050	0.046	0.046		0.002	0.065	0.065	0.108
H-49	0.050	0.046	0.046	0.002		0.065	0.065	0.110
O-N	0.063	0.060	0.060	0.068	0.068		0.003	0.113
O-143	0.063	0.060	0.060	0.068	0.068	0.003		0.113
GM-1	0.124	0.120	0.120	0.117	0.118	0.122	0.122	

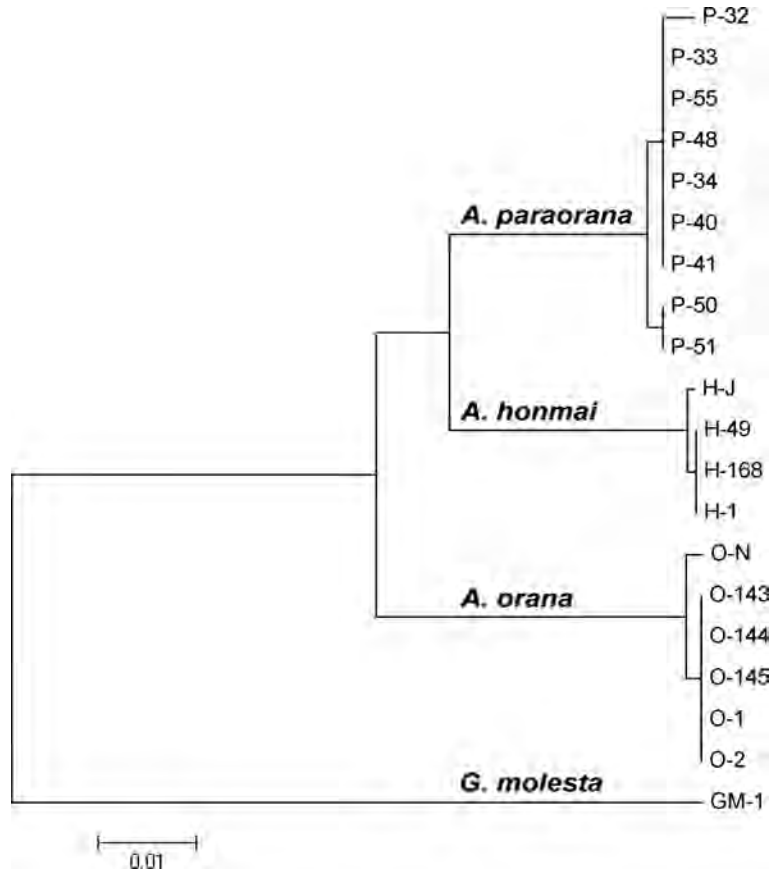


Figure 16. Phylogenetic tree based on neighbor-joining analysis. *Grapholita molesta* (GM-1) was used as an outgroup. Three species were grouped separately in the tree. Bootstrap proportion for the node for each species was 100%.

16.V.1997 (B.K. Byun); 1♀, same locality, 19.V.1997 (B.K. Byun); 1♂, same locality, 26.V.1997 (B.K. Byun); 1♂, same locality, 31.V.1997 (B.K. Byun); 1♂, same locality, 30.VI.1997 (B.K. Byun); 1♂, 1♀, same locality, 14.VII.1997 (B.K. Byun); 2♂, 1♀, same locality, 21.VII.1997 (B.K. Byun); 1♂, same locality, 27.IX.1997 (B.K. Byun); 1♂, Gwangneung, GG, 22. V. 1998 (B.K. Byun); 2♂, 2♀, same locality, 10. VI. 1998 (B.K. Byun) -gen. slide no. 341, 374; 2♂, same locality, 11. VI. 1999 (J.C. Sohn); 2♂, 1♀, same locality, 27. VII. 1998 (B.K. Byun); 1♂, same locality, 10. VIII. 1999 (B.K. Byun); 1♀, same locality, 13. VIII. 1998 (B.K. Byun); 1♀, same locality, 10. IX. 1998 (B.K. Byun) -gen. slide no. 342; 1♂, same locality, 10. IX. 1998 (B.K. Byun); 4♂, 3♀, Suweon, GG, 20.VII.1999 (J.M. Lee and K.S. Han)-gen. slide no. 357, 336, 338, 339, 361, 370; 1♂, Osan-si, GG, 6.X.1997 (B.K. Byun); 1♂, same locality, 27.IX.1997 (B.K. Byun); 2♂, Seomyun, Yangyang, GW, 4.VI.1987 (K.T. Park); 1♀, Hongcheon, Naemyeon, GW, 14.VIII.1987 (K.T. Park)-gen. slide no. 2486; 6♀, Unduryeong, Mt. Gyebang, GW, 13.VIII. 1995 (B.K. Byun)-gen. slide. no. 76, 77, 83; 6♂, 6♀, Seonghwan-eup, Cheonan-si, CN, 17.VII.2008, (C.Y. Yang)-gen. slide. no. 793, 794; 1♂, Gyeongju-si, GB, 6.VI.1996 (Y.H. Joo)-coll. HUNHM.

Distribution. Korea, Japan, and Europe.

Remarks. In Japan, this is an important pest on various fruit crops, especially on apples, and the larvae are voracious feeders, causing considerable damage to foliage, blossoms and fruits (Yasuda 1998). The existence of this European species in Korea has been recently noticed in a molecular study (Park et al. 2008) and a pheromonal study (Yang et al. 2009).

Molecular evidence

In addition to a part of the previously reported molecular data on COI (Park et al. 2008), we collected the data from various localities for the three species and compared all together. From these, some representatives are compared for their sequence similarity (Table 1). The p-distance among the three species were 4.4% and higher while the maximal intraspecific variation was 0.6% (Table 2). K2P-distances are similar to the p-distances.

The phylogenetic analyses using neighbor-joining resulted in a tree of which the topology confirmed specific distinction among the three species (Figure 16). The genus *Adoxophyes*, rather unique among the genera in Tortricinae, often causes identification problems due to the morphological similarities among species, but the three species can be easily distinguished using the COI barcode sequences. Although we know that the use of COI barcode sequences for the purpose of specific distinction can sometimes be problematic when the interspecific distance is lower than usual while the intraspecific distance is higher than usual, i.e., 1.6% of interspecific distance at minimum and 1.4% of intraspecific distance at maximum, our data in *Adoxophyes* species showed that the samples were clustered intraspecifically with less than 1% distance, yet separated into three main *Adoxophyes* species. Therefore the result supports species distinction by morphology as well as validating *A. paraorana* as a new species.

Notes

1. sa-gwa-ae-mo-mu-nui-ip-mal-i-na-bang.
2. cha-ae-mo-mu-nui-ip-mal-i-na-bang.
3. ae-mo-mu-nui-ip-mal-i-na-bang.

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