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Morphological diagnosis of six *Liriomyza* species (Diptera: Agromyzidae) of quarantine importance in Taiwan

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Abstract

Liriomyza leafminers comprise a pest group that causes both considerable economic losses and serious quarantine problems. In this study, morphological studies were performed to assist in the species identification and discrimination of six *Liriomyza* pest species of quarantine importance: *L. brassicae*, *L. bryoniae*, *L. chinensis*, *L. huidobrensis*, *L. sativae*, and *L. trifolii*. The discriminative ability of some traditional morphological characters, such as abdominal color patterns and male genitalia, was re-evaluated. In addition, electronmicrographic and geometric morphometric methods were introduced for separating different species. Illustrative plates of the preceding morphology were collated into mappings for further applications in quarantine inspection; some analyses and evaluations in separating similar species are also discussed in further detail. The results show that the abdominal color patterns can only separate two species from others; nevertheless, the ultrastructures of the thoracic microsetae and male genitalia are useful morphological characters to prevent misidentifications. On each species, the thoracic microsetae show their unique arrangement and pattern in both length and density; meanwhile, photography of male genitalia using a larger focal depth was also proven to be taxonomically valuable in practice. Although not all pairs between any two species have significant differences in wing shape, wing morphometric results do reveal that the most variant area of wing shape is located around the cross veins; this suggests that the morphology of this area might be easily and efficiently used for differentiating these species.

Key words: *Liriomyza*; morphology; ultrastructure; morphometrics; quarantine inspection

INTRODUCTION

Genus *Liriomyza* is one of the most intensively studied and well-documented pest groups in Agromyzidae. Parrella (1987) reviewed the literature on *Liriomyza* leafminers, and concluded that this pest group can impact crops in at least six ways: vectoring disease, destroying young seedlings, causing reductions in crop yields, causing "sunburning" of the fruit, reducing the aesthetic value of ornamental plants, and causing problems for plant quarantine. Historically, *Liriomyza* species were classified as minor pests, but in the early 1980s, the pest populations increased rapidly; some species, such as *L. trifolii* and *L. sativae*, developed insecticide resistance and threatened the chrysanthemum and celery industries in North America (Trumble, 1981; Parrella et al., 1984). Furthermore, they became serious worldwide pests due to human-mediated dispersal (Minkenberg, 1988). Parrella and Keil (1984) pointed out that the reasons for the sudden prominence of *Liriomyza* pests were: (1) taxonomic confusion, (2) failure of quarantine procedures, (3) lack of basic biological and ecological studies, and (4) patterns of insecticide use without regard to resistance development. In Taiwan, *Liriomyza* species were also reported to be causing damage to cultivated flowers and vegetables (Lee, 1986; Lee et al., 1990; Lin and Wang, 1992; Cheng, 1994). Meanwhile, some outbreak species have never been recorded in Taiwan before, and these are now believed to have been inadvertently introduced from other countries due to quarantine failure or plant smuggling (Wang and Lin, 1988; Shiao, 1991; Chien, 1997; Shiao and Wu, 2000). Similar situations have also occurred in nearby Asian areas, such as in mainland China and Japan (Kang, 1996; Iwasaki et al., 2000; Kasugai et al., 2001; Zhang et al., 2001).

Because of the increased volume of international trade of agricultural products, especially in flowers

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and vegetables, efficient detection and accurate species recognition of agromyzid pests on imported and exported crops have become increasingly important. Even though fully developed leafmines are readily detected at ports of entry by plant quarantine officials, the eggs, early larval instars, and young mines are otherwise minute and insignificant. Moreover, some species of *Liriomyza* adults have similar external morphology and overlapping host ranges, further complicating correct identification. Some attempts have been made to use contemporary techniques to distinguish *Liriomyza* species of economic importance. Zehnder et al. (1983) first proposed using scanning electronmicrography and isozyme electrophoresis to distinguish the three species of *L. brassicae*, *L. sativae*, and *L. trifolii*; Menken and Ulenberg (1986) performed allozymatic analysis of four economically important *Liriomyza* species; Oudman (1992) identified economically important *Liriomyza* species and their parasitoids using enzyme electrophoresis; while Collins (1996) also used isozyme electrophoresis to separate *L. huidobrensis* from other related *Liriomyza* species. Recently, Scheffer and Lewis (2001) provided molecular evidence of mtDNA for distinguishing different local populations of *L. huidobrensis*; Chiu et al. (2000a, b) used RAPD-PCR and isoenzyme electrophoretic methods to develop rapid diagnostic techniques for six *Liriomyza* species; and Scheffer et al. (2001) proposed PCR-RFLP methods to differentiate morphologically similar species of *L. huidobrensis* and *L. langei*. However, all of their materials must be identified in advance by morphology; nevertheless, Spencer and Steyskal (1986) mentioned that many groups of closely related species in some agromyzid genera cannot be reliably separated using external characters, and identification is only possible from examination of the male genitalia. This is a reminder that it is necessary to re-examine and evaluate the utility of the most fundamental morphological characters that have long been overlooked. To this goal, in this report, some traditional morphological, ultrastructural, and modern morphometric approaches based on adults of *Liriomyza* pest species are proposed and discussed in further detail.

In this paper, I evaluate the morphological discrimination of six *Liriomyza* species of quarantine importance. Among these six species, three (*L. huidobrensis*, *L. sativae*, and *L. trifolii*) are on the EPPO (European and Mediterranean Plant Protection Organization) A1 or A2 quarantine list (EPPO, 2002). The other three species are common pests of quarantine significance that have also frequently been reported to cause damage in some specific areas (Spencer, 1989).

MATERIALS AND METHODS

Insect specimens. The specimens used in this study were mainly from the collections of the Department of Entomology, National Taiwan University (NTU). In addition, specimens from Japan and from a laboratory colony in California (at UC Davis) and in Taipei were included in this study. Detailed specimen data are given in Table 1.

Traditional morphology. After being boiled in a 15% KOH solution for 5 min, male genitalia were dissected and examined under a stereomicroscope (Leica MZ125, Heerbrugg, Switzerland); genitalia were mounted on micro slides using Euparal, then photographed using a phase-contrast photomicrographic system (Olympus BH-2, PM-10AD, Tokyo, Japan). For obtaining genitalia images of better quality with larger focal depth, images captured from different focal planes were combined using the image processing software Auto-montage version 3.04 (Syncroscopy Division, Synoptics, Cambridge, U.K.) for some species. The abdomens were removed from male specimens, cleaned with KOH solution to eliminate the inside contents and mounted flat on micro slides. Abdominal color patterns were then recorded for each sample slide. The morphological terminology used in this article mainly follows that of Sasakawa (1961) and Spencer (1973).

Ultrastructures and measurement. Specimens for electronmicrography were dried naturally at room temperature; gold sputter-coated (using a Hitachi HUS-5GB High Vacuum Evaporator, Tokyo, Japan) specimens were examined and photographed at 20 kV (using a Hitachi S-520 SEM). To measure the length of thoracic microsetae, the coordinates of two endpoints of tip and base of each microseta were encoded from electronmicrographic image files by using the software TPSDIG version 1.31 (Rohlf, 2001a). Lengths of microsetae were then computed based on their coordinates and scale factors. Numbers of specimens used in this

Table 1. Source locality and host plant data for *Liriomyza* specimens

Species	Collecting localities	Host plants	Date	Collector	Identifier
L. brassicae	Taipei, Taiwan	*Brassica oleracea* L.	30-XII-1981	T. C. Hsu	S. F. Shiao
	Yushih, Nantou, Taiwan	*Pisum sativum* L.	22-XII-1990	S. F. Shiao	S. F. Shiao
L. bryoniae	Yuching, Tainan, Taiwan	*Citrullus vulgaris* Schrad. ex Eckl. & Zeyh.	4-I-1991	W. J. Wu	S. F. Shiao
	NTU colony, Taipei, Taiwan	*Brassica campestris* L.	1995–1996	S. F. Shiao	S. F. Shiao
	Naganuma, Hokkaido, Japan	*Gypsophila paniculata* L.	8-VI-1991	A. Iwasaki	A. Iwasaki
L. chinensis	Ilan City, Taiwan	*Allium fistulosum* L.	18-VIII-1990	W. J. Wu	S. F. Shiao
	Hualien City, Taiwan	*Allium odorum* L.	1-IV-1991	R. F. Chen	S. F. Shiao
L. huidobrensis	Wufeng, Taichung, Taiwan	*Lactuca sativa* L. var. *intybeca* Hort.	17-I-1999	L. Y. Chou	S. F. Shiao
	Neimen, Kaohsiung, Taiwan	*Luffa cylindrical* (L.) M. Roem	3-XII-1998	S. J. Chang	S. F. Shiao
	UC Davis colony, CA, USA	unknown	7-VII-1989	O. Minkenberg	O. Minkenberg
L. sativae	Wufeng, Taichung, Taiwan	*Phaseolus vulgaris* L.	7-VI-2000	J. C. Hsu	S. F. Shiao
	Hsinying, Tainan, Taiwan	*Lycopersicon esculentum* Mill.	9-II-2000	Y. C. Chiu	S. F. Shiao
L. trifolii	Tienwei, Changhua, Taiwan	*Gerbera jamesonii* Bolues ex Hook. f	13-XI-1989	S. F. Shiao	S. F. Shiao
	Hamamatsu, Shizuoka, Japan	*Lycopersicon esculentum* Mill.	V-1991	A. Iwasaki	A. Iwasaki
	UC Davis colony, CA, USA	unknown	7-X-1989	O. Minkenberg	O. Minkenberg

measuring study are noted in Table 3, three samples from randomly selected areas on the thorax of each specimen were used, and the measurements were averaged to minimize the measuring errors.

Wing morphometrics. Wing measurements were made using a phase-contrast microscope with a TV camera and a computer-based image enhancement system. Wings were removed from male adults and mounted on micro slides. Morphometrics of wing venation were measured by analyzing image files captured with a digital camera (DMC-1, Polaroid, Cambridge, MA, U.S.A.). Sixteen selected landmarks on a wing were digitized into coordinates using the software TPSDIG version 1.31 (Rohlf, 2001a) (for the selection, definition, and numbers of landmarks see Table 2). Each sample was digitized three times to reduce digitizing errors, and then the software of GRF (Rohlf and Slice, 1989) was used to superimpose those landmarks. Computations using the criterion of generalized least-squares fit (GLS) (Rohlf and Slice, 1990) were performed to reveal landmark

Table 2. Selection and definition of landmarks on wings of six *Liriomyza* species

Landmark no.	Location and definition
#1	branching point of C and Sc
#2	intersection of R_1 and C
#3	intersection of R_{2+3} and C
#4	intersection of R_{4+5} and C
#5	intersection of M_{1+2} and C
#6	intersection of M_{3+4} and wing margin
#7	intersection of Sc and R
#8	branching point of R_1 and Rs (R_{2+3}, R_{4+5})
#9	branching point of R_{2+3} and R_{4+5}
#10	intersection of inner cross vein (r-m) and R_{4+5}
#11	root (basal point) of M
#12	branching point of M_{1+2} and M_{3+4}
#13	intersection of inner cross vein (r-m) and M_{1+2}
#14	intersection of outer cross vein (m-m) and M_{1+2}
#15	intersection of outer cross vein (m-m) and M_{3+4}
#16	branching point of Cu+A

C, costa; Sc, subcosta; R, radius; M, media; Cu, cubitus; A, anal vein.

variations among all sample specimens. The output mean shapes (or reference shapes) of each species obtained by GLS were then used as input for thin-plate spline relative warp analysis (TPSRELW version 1.24) (Rohlf, 2001b). Ordination plotting and a minimum spanning tree based on average taxonomic distances for each species based on the results of the relative warp analysis were accomplished using the software NTSYSpc version 2.01b (Rohlf, 1997). MANOVA was then performed based on the new data W matrix and affine components (Rohlf, 1993), which were generated from the thin-plate-spline-based analysis, to see whether the overall shape information of wings varied significantly among species.

RESULTS

Color patterns on abdominal tergites

Over 1,300 male abdomen slides were examined (sample size for each species: *L. brassicae*=60; *L. bryoniae*=520; *L. chinensis*=50; *L. huidobrensis*=150; *L. sativae*=220; *L. trifolii*=350). Since no different abdominal color pattern was observed within the same species from different localities, the species of *L. bryoniae*, *L. huidobrensis* and *L. trifolii* used in this analysis were the three mixture samples of populations from Taiwan and Japan or from Taiwan and USA (see Table 1 for the source localities of these specimens). According to the results here (Fig. 1), the yellow-tinged middle furrows on the central line of the abdominal tergites were shown to have a much higher value for dividing some species, and three types of color patterns were determined to exist in these six species. The principal differences were basically caused by longer or shorter yellow middle furrows on those six visible abdominal tergites, especially as to which or how many tergal segments are divided by the yellow furrow. The most common type, which only has the second visible tergite divided by the yellow middle furrow (Fig. 1B, D, E), includes the three species of *L. brassicae*, *L. huidobrensis*, and *L. sativae*. Another two species, *Liriomyza bryoniae* and *L. trifolii*, have otherwise longer yellow furrows with two and four divided tergites, respectively (Fig. 1A, C). The first tergite is not included in this comparison, since it is too narrow to see whether it is divided by the yellow furrow or not, and also some variations occur within the same

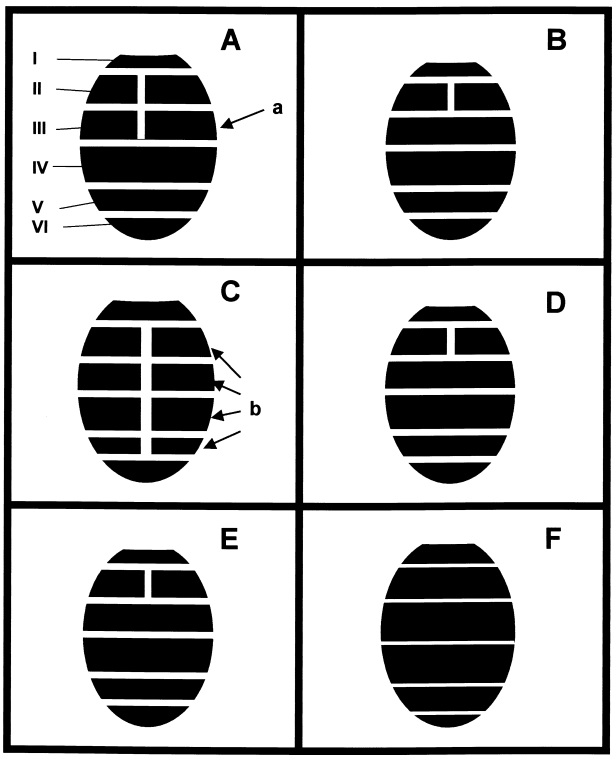

Fig. 1. Diagrams of abdominal color patterns of six *Liriomyza* species. A, *L. bryoniae*; B, *L. huidobrensis*; C, *L. trifolii*; D, *L. sativae*; E, *L. brassicae*; F, *L. chinensis*. I to VI indicate the first to sixth visible abdominal tergites; a, the third visible tergite is divided by the yellow middle furrow in *L. bryoniae*; b, the second to fifth tergites are divided by the yellow middle furrow in *L. trifolii*.

species. On the other hand, *L. chinensis* is a special species which shows no obvious color pattern and even has a pale gray mesoscutellum on the thorax, not yellow as in the preceding species. Generally speaking, *L. trifolii* has the most obvious color pattern with up to four divided tergites (Fig. 1b), and the yellow middle furrow of *L. bryoniae* stably appears on the 3rd visible abdominal tergite (Fig. 1a).

Thoracic microsetae

The ultrastructural characters, especially the tiny background setae on thoracic tergite (mesonotum), of six *Liriomyza* pest species were herein examined. The results show that not only the density but also the length and shapes of those microsetae differ among species (Fig. 2). Table 3 reveals that *L. brassicae* has the shortest microsetae, and *L. chinensis* has the longest ones; and except for *L. bryoniae* and *L. sativae*, thoracic microsetae show significant differences in length among the different

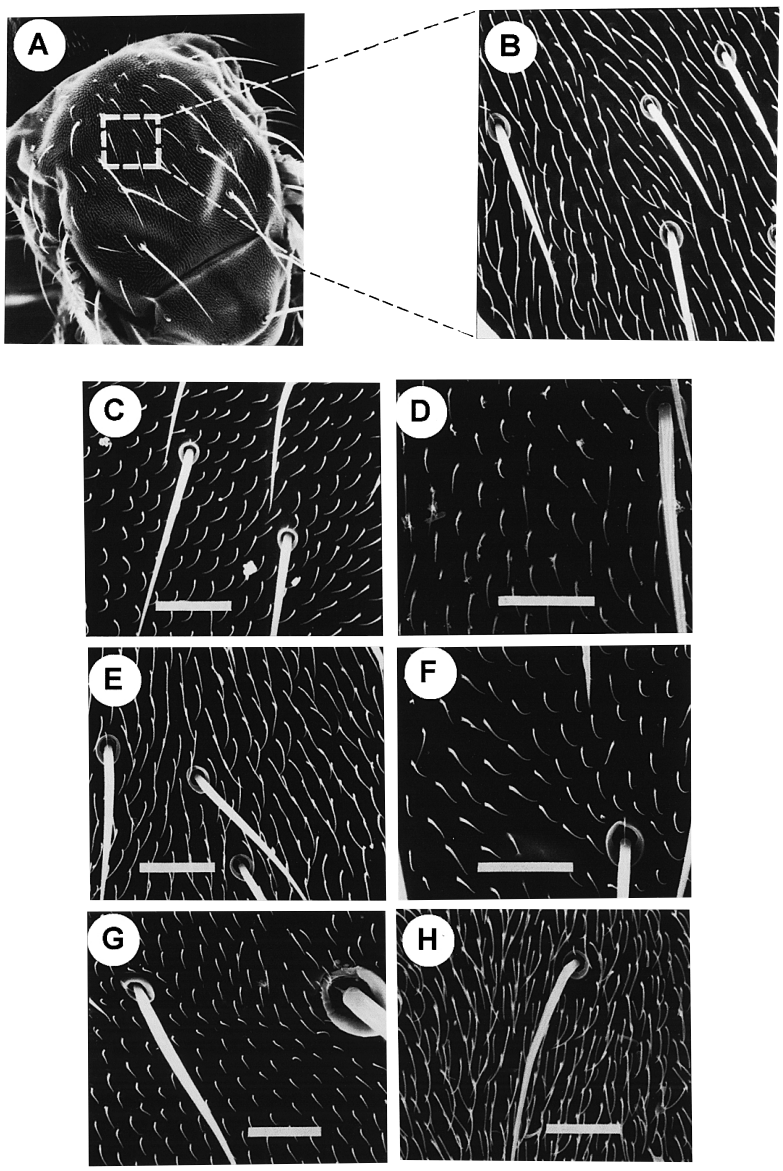

Fig. 2. Arrangements of thoracic microsetae of six *Liriomyza* species. A and B indicate the location of microsetae on the thoracic tergite and their enlargement; C, *L. bryoniae*; D, *L. huidobrensis*; E, *L. trifolii*; F, *L. sativae*; G, *L. brassicae*; H, *L. chinensis*. Scale bar=20 μm.

species. In addition, the density of the microsetae can also be used as a reliable diagnostic character for dividing these six pest species (Table 3). Moreover, the shapes of the microsetae themselves differ among the species under larger magnification (Fig. 3). On the other hand, to further examine the discriminative ability of these measurements using in species identification, General Linear Discriminant Analysis was preformed to test the percentage of correct identification. The results are given in Table 4. When individually using the lengths or the densities of microsetae as the diagnostic character, the total percentage of correct identification is about 74.3% and 73.8% respectively; and if using both characters, the correct identification percentage will be further increased up to 80%.

Wing morphometrics

The results of least-squares superimposition of 16 landmarks for a total of 297 wing specimens in six *Liriomyza* species clearly show the relative variations for each landmark among the different samples (Fig. 4). Among the 16 selected landmarks, landmarks #2, #3, and #6 present larger

Table 3. Comparisons of length and density of thoracic microsetae for six *Liriomyza* species. All samples belong to Taiwanese populations, for detailed specimen data see Table 1

Species	Length		Density	
	n	Mean±SD (μm)	*n*	No. per 40 μm^2 Mean±SD
L. brassicae	100	4.13±0.07 a	30	52.33±2.05 c
L. bryoniae	50	5.01±0.07 b	30	46.75±4.97 bc
L. chinensis	100	9.60±0.17 e	30	97.50±2.08 e
L. huidobrensis	50	5.58±0.15 c	30	36.50±4.87 a
L. sativae	100	5.00±0.13 b	30	42.00±2.78 ab
L. trifolii	50	8.21±0.10 d	30	66.50±3.48 d

n, sample size, numbers of specimens; means followed by different letters significantly differ at α=0.05 (by Scheffé's test).

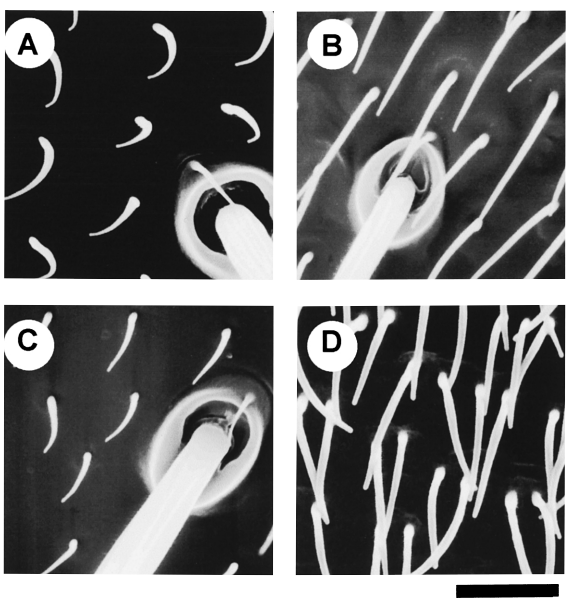

Fig. 3. Local enlargements of thoracic microsetae of *Liriomyza* species. A, *L. bryoniae*; B, *L. trifolii*; C, *L. brassicae*; D, *L. chinensis*. Scale bar=6 μm.

Table 4. Percentages of correct identification[a] using characters of microseta measurements for *Liriomyza* species. All samples belong to Taiwanese populations, for detailed specimen data see Tables 1 and 3

Species	Correct percentage using length (%)	Correct percentage using density (%)	Correct percentage using both length and density (%)
L. brassicae	86	93	100
L. bryoniae	NC[b]	37	70
L. chinensis	74	100	100
L. huidobrensis	58	60	57
L. sativae	NC	63	60
L. trifolii	71	100	100
Total[c]	74.3	73.8	80.0

[a] Based on classification matrix of General Linear Discriminant Analysis (tolerance limit=0.01).
[b] No significant difference based on ANOVA test, and was excluded from this analysis.
[c] Summarized results of correct identification rates for all species by using the characters.

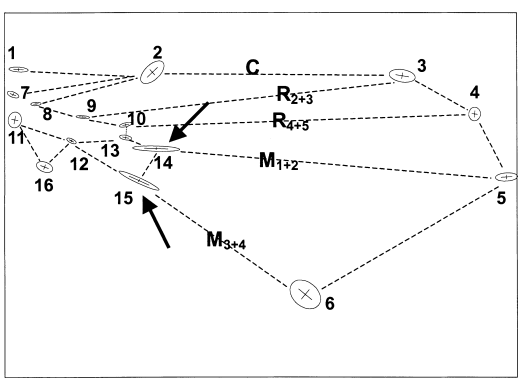

Fig. 4. Results of generalized least-squares analysis for superimposing 16 landmarks on the wings of six *Liriomyza* species. First and second principal component axes with one standard deviation length and equal frequency ellipses of two standard deviations are presented for each landmark. Arrows indicate the location of landmarks (#14 and #15) on the outer cross vein that have directional variation.

variability, having larger elliptical areas of two standard deviations as shown in Fig. 4. Otherwise, landmarks #14 and #15 present special directional variability as revealed by the flatter ellipses. This indicates that special variations occur on the outer cross vein (landmarks #14 and #15 are on both ends of the outer cross vein); According to the longer first principal component (PCA) axes and the shorter second PCA axes of one standard deviation, I believe that the landmark variations appear mostly along the longitudinal vein of M_{1+2} and M_{3+4}. Although landmarks #2, #3, and #6 on the wing margin have large variability, the resultant areas of rounder ellipses of two standard deviations suggest that these differences are probably caused by some random and non-directional changes.

The thin-plate spline relative warp analysis was computed to summarize wing variations among

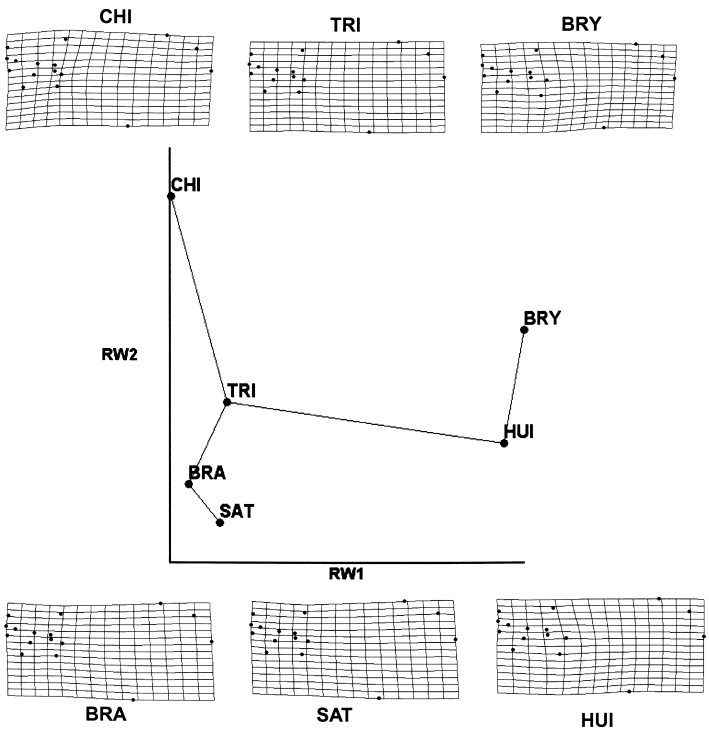

Fig. 5. The minimum spanning tree superimposed onto the scatterplot of the second relative warp (RW2) against the first relative warp (RW1) of wing shapes of six *Liriomyza* species. Shape changes of each species are represented by deformation grids. The first and second axes account for 57.6% and 30.2% of the total variance, respectively. BRA, *L. brassicae*; BRY, *L. bryoniae*; CHI, *L. chinensis*; HUD, *L. huidobrensis*; SAT, *L. sativae*; TRI, *L. trifolii*.

species in as few dimensions as possible. For taxonomic studies, the scaling parameter, α, in relative warp analysis was set to 0 as recommended by Rohlf (1993); this becomes a principal components analysis of the covariance matrix of the partial warp scores. Results show that different shape changes occur mainly on the central area of the wing, especially at the location of the cross veins (r-m and m-m cross veins) (Fig. 5). By superimposing the minimum spanning tree onto the scatterplot of the first and second relative warp axes, we can clearly see the relative recency among these six species. According to Fig. 5, the minimum spanning tree suggests that *L. trifolii*, *L. brassicae*, and *L. sativae* have similar overall wing shapes, while the wing of *L. chinensis* has a relatively different shape that is on the far terminal of this tree. In addition, the *L. huidobrensis* and *L. bryoniae* were closely connected in this tree, which shows their close relationship. The first and second relative warps account for 57.6% and 30.2% of the total wing variations, respectively.

One-way MANOVA was then performed using the W matrix (Rohlf, 1993) (the matrix of partial warp scores, the projection of all the specimens onto the partial warps, which can be treated as a decomposition of shape information of non-affine changes for each species) and the affine components to determine if wing shapes vary significantly among different species. The multiple comparison results of both affine and non-affine components show that, except among *L. trifolii*, *L. brassicae*, and *L. sativae* (*L. trifolii* versus *L. brassicae*, $p=0.43$; *L. trifolii* versus *L. sativae*, $p=0.55$; *L. brassicae* versus *L. sativae*, $p=0.18$), all pairs between any two species have significant shape differences (MANOVA test, $p<0.05$, based on Hotelling's T^2 values).

Male genitalia

Male genitalia of six *Liriomyza* species were dissected, cleaned, photographed and software-combined to obtain the resultant photography with a larger focal depth, as in Figs. 6 and 7. I found it is hard to conclude whether the morphologies of epandrium, surstylus, hypandrium, phallapodeme

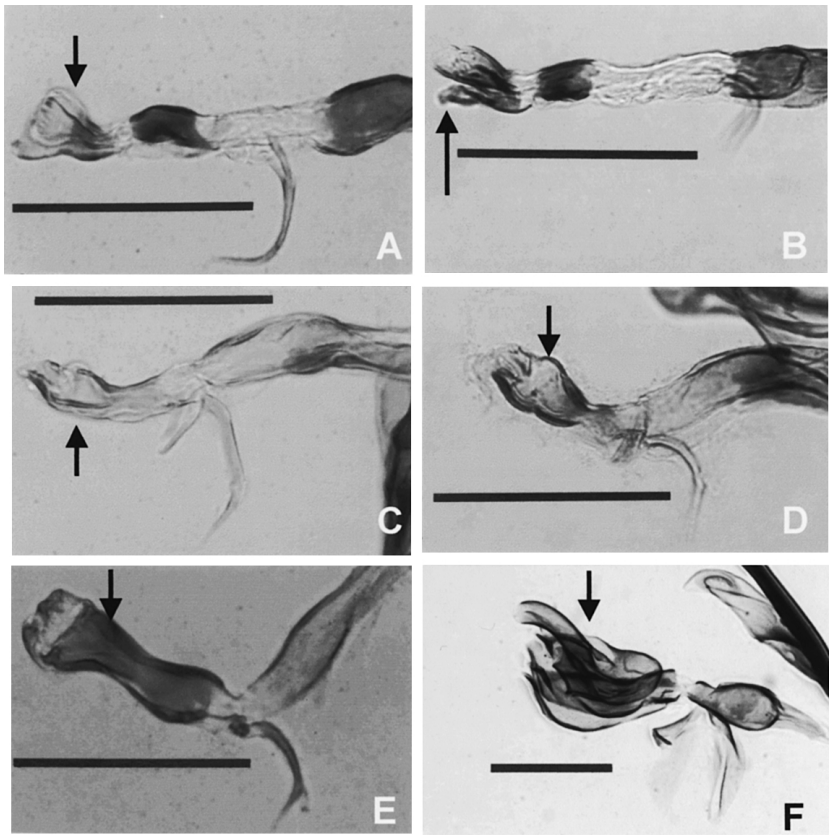

Fig. 6. Photo plates of the phalluses of six *Liriomyza* species, lateral view. A, *L. bryoniae*; B, *L. huidobrensis*; C, *L. trifolii*; D, *L. sativae*; E, *L. brassicae*; F, *L. chinensis*. Arrows indicate the distiphallus. Scale bar=0.1 mm.

and cerci have any discriminative value for taxonomic or identification purposes. In contrast, the phallus (or aedeagus) in most species is highly evolved and characteristic. The phallus consists of four sections: the basiphallus, mesophallus, hypophallus, and distiphallus. According to the photomicrographic results in Figs. 6 and 7, one can see that the distiphallus has the most conspicuous shape, and this is probably the most useful character in identifying *Liriomyza* species. However, the problem is when one treats closely related species, like the species mentioned in this paper, the morphology of the distiphallus is still easily confused without careful examination. There are two pairs of species in my studies which have similarly shaped phalluses. *L. bryoniae* and *L. huidobrensis* are one of the resembling pairs (Figs. 6A, B and 7A, B) in which the distiphallus is similar, especially from the lateral view. But careful examination reveals that the ventral views of the distiphallus actually differ, in that *L. bryoniae* has a more-rounded bowl-shaped distiphallus when compare with that of *L. huidobrensis* (Fig. 7A, B). In addition, the mesophalluses of these two species have different sclerotized areas: the mesophallus of *L. huidobrensis* has a pair of parallel, highly-sclerotized stripes on lateral margins from ventral view (Fig. 7B), although they are obscure without a properly prepared sample. The other pair of *L. trifolii* and *L. sativae* is much more difficult to separate; differences of these two species mainly appear on the distiphallus (Figs. 6C, D and 7C, D). However, differences between these two species are quite hard to put into words, and the only ones I am able to recognize are the different degrees of sclerotization and minor shape changes on the distiphallus. In this case, the lateral views (Fig. 6C, D) seem much easier to use in separating these two species. In general, the distiphallus of *L. sativae* comprises a relatively larger area in the phallus and usually has a more distinct sclerotized shape when compared with that of *L. trifolii*.

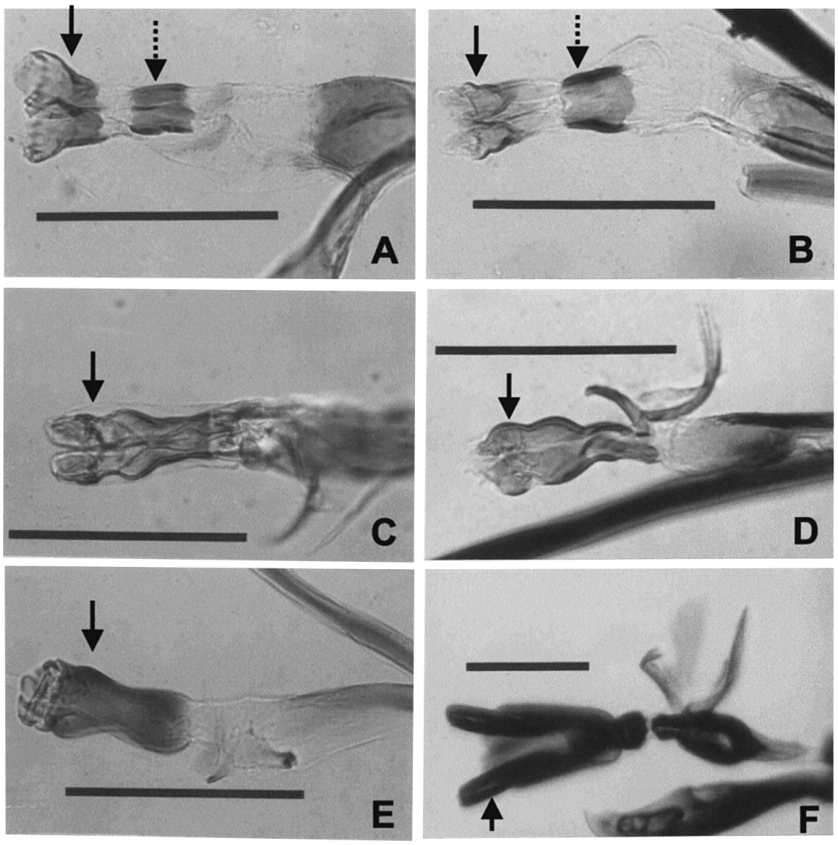

Fig. 7. Photo plates of the phalluses of six *Liriomyza* species, ventral view. A, *L. bryoniae*; B, *L. huidobrensis*; C, *L. trifolii*; D, *L. sativae*; E, *L. brassicae*; F, *L. chinensis*. Solid arrows indicate the location of distiphallus; dotted arrows indicate the mesophallus in *L. bryoniae* and *L. huidobrensis*. Scale bar=0.1 mm.

DISCUSSION

Color patterns on abdominal tergites

Most *Liriomyza* adults can be readily recognized from other agromyzid genera by the appearances of the yellow frons and scutellum, but these color appearances are merely the early concept of this genus and not consistent characters for taxonomic identification (Spencer and Steyskal, 1986). However, almost all *Liriomyza* pest species have such special color patterns, which also provide an obvious external morphological character for preliminary recognition of members of this genus in the field. In the past, color patterns on the mesonotum were proposed to help recognize some *Liriomyza* species (Spencer and Steyskal, 1986). Among these pest species, the abdominal tergites also show these color patterns, and they are somewhat more characteristic than on the frons, mesonotum, and scutellum. Unfortunately, only a few studies have provided descriptions of the coloration. Sasakawa (1960, 1961) characterized the tergal color patterns by depicting the yellow area of the lateral and caudal margins on each tergal segment, but neither drawings nor detailed descriptions were provided. In this study, some species-specific patterns were discovered. Although abdominal color patterns can provide an easy, rapid tool for species diagnosis, they still cannot efficiently differentiate every species, especially when those species with the same color pattern are encountered. Meanwhile, the pattern mapping proposed here is only suitable for application in male adults; female adults in my examinations presented much greater ambiguity among species. Since some examined specimens were already deposited in the museum over 20 years, I believe the time of collection won't affect the character of color patterns. However, in reality, these kinds of abdominal color patterns are much more easily recognized using fresh samples; for dry specimens, the telescoped abdomens are usually shortened and shriveled, so they must be re-

moved from specimens, stretched, and smoothly flattened on a slide for better observations.

Thoracic microsetae

Zehnder et al. (1983) first proposed the morphology of thoracic microsetae for the diagnostic discrimination of *Liriomyza* species using scanning electron microscopy; they concluded that only *L. trifolii* could be separated from the other two species (*L. sativae* and *L. brassicae*) due to its dense covering of microsetae. They undoubtedly provided a useful direction for exploring new morphological characters using ultrastructure, and their methods also meet some requirements for rapid diagnosis in quarantine procedures, such as saving time and effort in identification by a non-expert. However, no detailed, further study was subsequently conducted. As previously mentioned, the results of the ultrastructural characters showed that not only the density but also the length of the thoracic microsetae differ among *Liriomyza* species; and simultaneously using the lengths and the densities of microsetae as the characters will further increase the identification accuracy. According to the enlarged photography in Fig. 3, the different arrangements and appearances of the thoracic microsetae are largely due to the different morphologies of the microsetae themselves, so they were also proven unambiguous and reliable and not the result of artifacts or parallax effects. Therefore, I believe these kinds of microseta mappings have great potential for application in quarantine inspections.

Wing morphometrics

Traditional wing characters of the Agromyzidae include costa ending points, length comparisons between the last section of M_{3+4} and the penultimate one, and the position of the inner cross-vein (r-m) on the discal cell (Spencer, 1973). Sasakawa (1961) used other additional measurements to describe the wing venation, such as the length proportion of the 2nd to 4th costal sections, a length comparison of the ultimate section of M_{1+2} with the penultimate one, and a length comparison of the outer cross vein (m-m) with the penultimate section of M_{1+2}. Although some of the preceding wing characters can partially describe venation differences, and some proportions can exclude the size effects from shape information, those traditional measurements still cannot meet the requirements for describing precise and complete information of wing shape and venation. Modern morphometrics uses a more direct and intuitive way by superimposing some encoded landmark coordinates to solve this problem (Rohlf and Slice, 1990). After rotation, translation, and scaling, one can align those landmarks as closely as possible, and see differences among specimens. Some interesting results were discovered by superimposing the wing shapes of *Liriomyza* species in this study: the special variations occurred on cross vein as described in the results, indicating their potential usage in identification purposes.

Although the results of minimum spanning tree and deformation grids do not appear to be developed enough to be readily applied in identifying *Liriomyza* species, they do clearly reveal the wing shape differences and their relationships among different species. The thin-plate-spline-based analysis (Thompson, 1917; Bookstein, 1989, 1991; Rohlf and Marcus, 1993) is a precise tool for exploring shape variations, and there are at least two advantages: its capacity to discern shape differences and the potential to graphically illustrate shape variation, as pointed out by Adams and Funk (1997). Complete wing shape information can therefore be compared and analyzed in this study, so it is worth further development in applications for identifying species, especially when used to detect minor shape differences between closely related species.

Male genitalia

Male genitalia probably comprise the most important taxonomic character in the Agromyzidae since Nowakowski (1962), and they subsequently were generally adopted as a major character by most leading specialists, such as Spencer (1990), Sasakawa (1963), Griffiths (1974), and Tschirnhaus (1971). Nevertheless, most of these characters are presented by means of drawings, and only a few photos (e.g., Tschirnhaus, 1981) can be found in the literature. Genitalia drawings are somewhat difficult to interpret, as different authors use different ways to express the genital characters, and the correct species identification always relies strongly on the quality, precision, and details of the drawings. Spencer (1992) mentioned that Sasakawa's drawings of genitalia are of the highest quality, and

are without question the best provided by any specialist on this family. This also implies that he considered the quality of drawings to be of importance in identifying agromyzid species. However, those genitalia drawings are more or less personalized and somewhat difficult to compare with actual dissected objects, especially for a beginner. For example, some authors use outline drawings to represent overall shapes; some prefer dotted drawings to emphasize the different areas or various degrees of sclerotization of the hard parts of the genitalia. I believe that using photography with a larger focal depth can overcome these defects of using traditional photos and also avoid misjudgement by reading descriptions or drawings.

As previously mentioned, male genitalia have become a most important discriminative character. Spencer and Steyskal (1986) have mentioned that illustrations in side, ventral, or both views will suffice to permit immediate identification even at the species level. I agree with their point and think that more detailed examinations are needed especially in dividing some similar species. However, there are limitations of separating a species from closely related ones only by minor morphological differences, and there is still a need for further evidence and detailed studies from more local areas to re-evaluate this. At least, the genitalia photo plates of the six *Liriomyza* species presented here are in good agreement with the current species definitions in the literature without local morphological variation (Sasakawa, 1960, 1961, 1963; Spencer, 1973, 1990), and could be reliably used in assisting species recognition.

Although traditional morphologies have long been considered subjective, more advanced morphological methods and instruments are now available for re-evaluating them in taxonomy, and furthermore, for exploring new morphological characters. In this paper, morphological characters of *Liriomyza* species are examined and evaluated; the purpose was to consider the use of morphological approaches in quarantine examination for a rapid and more-accurate species judgment. Using electronmicrography and computer-aided image enhancement systems, we can produce images of better quality to assist in identification; using modern morphometric analysis, we can precisely locate and quantify differences in wing shape and venation. In conclusion, there is no single character which can conveniently and successfully differentiate every *Liriomyza* species, even among genitalia characters. This is partially caused by the highly homogenous morphology within this group. Nevertheless, the highly similar morphology in the *Liriomyza* pest group does not match its diversified ecology; different species with different host preferences, parasite complexes, and pesticide resistances do deeply affect quarantine and control policies. This preliminary species discrimination is only the first basic step in assisting ongoing related studies to look into these problems.

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