

行政院國家科學委員會專題研究計畫成果報告

近緣種之多食性潛蠅形態進化與系統學研究

Morphological evolution and systematics studies on closely related polyphagous agromyzid species

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一、 中文摘要

傳統分類部份持續整理台灣地區名錄，蒐集台灣產潛蠅科種類並重新描述及提供較為詳盡之特徵資料；並研究以相對扭曲(Relative warp)定序法或聚類分析以潛蠅翅脈為主作系統學或進化學等相關推論。本年度主要以分析斑潛蠅害蟲形態鑑別及潛蠅科系統學關係為主要工作內容；斑潛蠅(*Liriomyza*)是臺灣地區近年來新崛起的蔬菜及花卉重要害蟲，部分種類亦被認為是經由進口所導致的新侵入害蟲；然而不論在檢疫與防疫的研究與應用上，種類的判定與區別一直困擾著研究與檢防疫工作者。斑潛蠅由於屬內形態同質性太高且寄主植物範圍廣泛重疊，再加上競爭取代現象頻繁，因此在國內外種類遭誤判的情況相當普遍；如此勢必嚴重影響檢防疫工作之精確性與研究結果的可靠性。本研究針對臺灣產6種主要斑潛蠅害蟲(同時也是重要的檢防疫對象害蟲)，包括：白菜斑潛蠅(*L. brassicae*)、蕃茄斑潛蠅(*L. bryoniae*)、蔥斑潛蠅(*L. chinensis*)、南美斑潛蠅(*L. huidobrensis*)、蔬菜斑潛蠅(*L. sativae*)及非洲菊斑潛蠅(*L. trifolii*)等作形態區辨之報導；目的在尋求簡易快速的形態區辨特徵，提供非分類學之相關工作人員應用參考。除討論雄蟲外生殖器特徵外，結果亦顯示在以有限的害蟲種類為對象下，腹部背板斑紋、翅脈形態測量及電顯局部放大之微毛列等特徵，可以在判定特定種類上有相當程度的應用價值。

關鍵詞：形態學、形態測量學，系統分類學，潛蠅科，雙翅目。

Abstract

Liriomyza leafminers is a pest group that causes not only the considerable economic losses but also the serious quarantine problems. Due to the increased volume of international trade of agricultural products and the morphological similarities of the closely related species in this pest group, efficient and accurate pest species recognition on imported or exported crops becomes more and more important; and this is probably the only way to prevent them from outspreading by human-mediated dispersal. Therefore, morphological studies were performed to assist species identification and discrimination of six *Liriomyza* pest species of quarantine importance, which include *L. brassicae*, *L. bryoniae*, *L. chinensis*, *L. huidobrensis*, *L. sativae*, and *L. trifolii*. Some traditional morphological characters, such as abdominal color patterns and male genitalia were re-evaluated for their discriminative ability. Besides, the electronmicrography and geometric morphometric methods were introduced to use in separating different species. Illustrative plates of the preceding morphology were collected into mappings for further applications in quarantine inspection; some analyses and evaluations in separating similar species were also discussed in further detail. The results show that the abdominal color patterns can only separate two species from others; nevertheless, the ultra-structure of thoracic microsetae and male genitalia are useful morphological characters to prevent

misidentifications. Thoracic microsetae show their unique arrangement and pattern on each species both in length and in density; meanwhile, the photography of male genitalia, which have larger focal depth, was also proven taxonomically valuable in practice. Although not all pairs between any two species have significant differences in wing shapes, wing morphometric results do reveal that the most variant area of wing shape is located around the cross veins; this suggests that the morphology on this area might be used easily and efficiently in dividing different species.

Keywords: Morphometrics, Systematics, Agromyzidae, Diptera.

二、緣由與目的

潛蠅科昆蟲原本只是農田的次要害蟲，但其害蟲地位在過去 20 年間有了劇烈的變化；從 80 年代大發生到因人為攜帶所引發的植物檢疫問題及至目前的入侵、競爭及取代的種種現象，使得我們對這些具經濟重要性的昆蟲，其行為、生態甚至分類、進化的基礎生物學問題有了更大的興趣。潛蠅科是無瓣類雙翅目中種類數較多的一個大類群；有趣的是僅有非常少數的種類在進化過程中衍生出多食的習性，其中更有多數種類集中在斑潛蠅(*Liriomyza*)屬中；這些種類也都是我們所熟知的害蟲。雖然斑潛蠅的經濟重要性極高，但分類鑑定的工作卻常常出錯；而這種鑑定錯誤所造成防治方針偏差的狀況到目前仍是一大困擾。1980 年代美國加州斑潛蠅大發生時，分類上的混淆就是一個例子。不過由實際分類的工作上來看，斑潛蠅在種級分類上的區辨的確並不容易；由於屬的同質性高，因此大部份的種類並不容易直接從外部形態來區分；目前分類學者多半採用穩定性較高的雄蟲生殖器作為分類依據。當兩種或兩種以上的雜食性種類同時並存於一個區域時，競爭、取代現象不斷發生，而此時正確地判斷出種類，往往是防治成功與否的第一個關鍵；Parrella 及 Keil (1983) 就曾指出斑潛蠅大發生及防治

的主要原因及盲點首在於學者們對種類鑑定上的混淆。

三、研究方法與進行步驟

主要標本來源為台大昆蟲系標本收藏 (by Tung-Ching Hsu (T. C. Hsu) and Wen-Jer Wu (W. J. Wu) during early 1980's and 1990's)，及本研究室近年的標本收藏 (by Liang-Yih Chou (L. Y. Chou), Yi-Chung Chiu (Y. C. Chiu), Shu-Chen Chang (S. J. Chang), and the author (S. F. Shiao))。除此之外並加入日本 (北海道)、美國 (UC Davis, California) 及台灣 (台大) 等三地之實驗室飼養品系為試驗材料。

雄蟲生殖器以 15% KOH 煮沸後解剖並鏡檢 (Leica MZ125, Heerbrugg, Switzerland) 後；以 Euparal 封片及以位相差顯微鏡攝影 (Olympus BH-2, PM-10AD, Tokyo, Japan)。為使外生殖器攝影得到更高之景深，以“Auto-montage version 3.04” (Syncroscopy division, Synoptics Ltd., Cambridge, U.K.) 影像處理軟體，作不同焦距平面影像之合成處理。

電子顯微鏡標本處理，採標本自然陰乾方式後鍍膜 (by Hitachi HUS-5GB High Vacuum Evaporator, Tokyo, Japan)；並在 20 KV 下作觀察攝影 (by Hitachi S-520 SEM, Tokyo, Japan)。

翅脈測量採用位相差顯微鏡 (Olympus BH-2, PM-10AD, Tokyo, Japan) 配合數位攝影機 (DMC-1, Polaroid, MA) 及電腦影像處理。翅脈地標點法之分析流程：

(1) 將各種類翅脈以影像檔方式儲存於電腦中；影像主要來源包括：

(i) 採集或誘集標本：將翅拔下，以 Euparal 平封於玻片中，利用攝影機及影像軟體以 TIFF 或 BMP 格式儲存。

(ii) 文獻繪圖：將繪有翅脈之文獻以掃描器掃描並配合軟體儲存成 TIFF 或 BMP 檔。主要的圖片來源包括：Sasakawa (1961)、Spencer (1973, 1986, 1987)、Shiao and Wu (1995a, 1996)、蕭 (1991)、蕭及吳 (1996) 等。

(2) 強化處理圖像檔之對比及亮度，使其易

於地標點之選取定位。

(3)地標點的選取及定義

共選取 16 個 (地標點作為分析資料; 選取的原則為儘可能選取所有翅脈交點以完整脈相之資訊; 翅脈交點依據地標點分類定義應可視為第 1 型地標點 (Type I landmark) (Bookstein *et al.*, 1985), 位置選取上較無爭議。

(4)數位化地標點座標錄取, 採用“TPSDIG version 1.31” (Rohlf, 2001a)軟體, 以純文字檔方式存取座標值。每一標本 (影像檔) 重覆 3 次錄取座標值, 以平均值座標作為最後之分析資料來源, 目的為減低數位化取點時之人為誤差。

(5)取得地標點後的資料最直接的處理就是以重疊法 (Superimposition) 重合各樣本以觀察及比較各地標點或個體之變異; 目前的重疊法主要使用最小平方和法則 “generalized least-squares fit” (GLS) (Rohlf and Slice, 1990)。

(6)再以薄版曲線及其他相關分析, 以更清楚瞭解變形 (Deformation) 的情況; 主要使用 “相對扭曲” 分析 (TPSRELW version 1.24) (Rohlf 2001b) 及加權矩陣 “W matrix” 及仿射因子 (affine components (Rohlf, 1993)) 之形狀因子以 UPGMA 聚類分析 (“NTSYSpc version 2.01b” (Rohlf 1997)), 表達各樣本的相似程度。

四、結果與討論

Color patterns on abdominal tergites:

Most of the *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 the consistent character for taxonomic identification (Spencer and Steyskal 1986). However, almost all the *Liriomyza* pest species have those kinds of special color patterns, and that also provided as an obvious external morphological character for preliminary recognition of the members in this genus in fields. In past, color patterns on mesonotum have been proposed to help recognizing some *Liriomyza* species

(Spencer & Steyskal 1986). Among these pest species, the abdominal tergites show these color patterns as well, and somehow more characteristic than on frons, mesonotum, and scutellum. Unfortunately, only few literatures have provided such descriptions of the coloration. Sasakawa (1960, 1961) characterized the tergal color patterns by depicting the yellow area of lateral and caudal margins on each tergal segment, but neither drawing nor detailed description has been provided. According to the results here (Fig. 1), the yellow-tinged middle furrows on the central line of abdominal tergites were revealed with much higher value for dividing some species; and three types of color patterns can be concluded in these six species. The principal differences are basically caused by lengthen or shorten of the yellow middle furrows on those six visible abdominal tergites; especially on 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 yellow middle furrow (Fig. 1-B, D, E), including three species of *L. brassicae*, *L. huidobrensis*, and *L. sativae*. Another two species of *Liriomyza bryoniae* and *L. trifolii* have otherwise longer yellow furrows with two and four divided tergites, respectively (Fig. 1-A, 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 there are some variations occur within the same species. On the other hand, *L. chinensis* is a special species which shows no obvious color pattern and even has the scutellum pale gray but not yellow as the preceding species. Although abdominal color patterns can be provided as an easy and rapid tool for species diagnosis, it still cannot efficiently divide every species, especially when treated with those three species that have the same common type of the color patterns. Generally speaking, *L. trifolii* has the most obvious color pattern which has up to four divided tergites (Fig. 1b), and *L. bryoniae* has the yellow middle furrow stably appeared on 3rd visible abdominal tergites (Fig. 1a). Meanwhile,

the pattern mapping proposed here is only suitable for applying in male adults, female adults in my examinations presented much ambiguous among species. In addition, the application reality of using these kinds of abdominal color patterns is that they are much easily recognized by using fresh samples; for those dry specimens, the telescoped abdomens are usually shortened and shriveled, so they must be removed from specimens, stretching and smoothly flattened on a slide for better observations.

Thoracic microsetae:

Zehnder et al. (1983) firstly proposed the morphology of thoracic microsetae for diagnostic discrimination of *Liriomyza* species by using scanning electron-microscopy, they concluded that only *L. trifolii* could be separated from other two species (*L. sativae* and *L. brassicae*) due to its dense covering of microsetae. They undoubtedly provided an useful direction for exploring new morphological characters by using ultrastructure; and their methods also meet some requirements for rapid diagnosis in quarantine procedures, such as time- and effort-saving in identification for a non-expert. Unfortunately, no detailed and further study was conducted afterward. I herein reexamine those ultra-structural characters of six *Liriomyza* pest species, and the results show not only the density but also the length and shapes of microsetae are different among them (Fig. 2). Table 3 reveals that *L. brassicae* has shortest microsetae and *L. chinensis* has longest ones; and except for *L. bryoniae* and *L. sativae*, thoracic microsetae have significant differences in length among different species. Meanwhile, the density of microsetae can also be used as a reliable diagnosis character for dividing these six pest species (Table 3). Moreover, the shapes of microsetae themselves are different among species under larger magnification (Fig. 3). This means that the preceding different appearances are largely due to the different morphology of microsetae

themselves; and in some ways, implies that the differences are neither caused by artifact during the procedure of sample preparation nor by parallax from different angles in observation. Therefore, I believe these kinds of microseta mappings have great potential for applying in quarantine inspections in the future.

Wing morphometrics:

Traditional wing characters of Agromyzidae include costa ending points, length comparisons between last section of M_{3+4} with penultimate, and the position of inner cross-vein (r-m) on discal cell (Spencer 1973). Sasakawa (1961) has used more other measurements in describing wing venation, such as the length proportion of 2nd to 4th costal sections, length comparison of ultimate section of M_{1+2} with penultimate, and length comparison of outer cross vein (m-m) with the penultimate section of M_{1+2} . Although some of the preceding wing characters can partially describe the venation differences, and some proportions can exclude the size effects from shape information; those traditional measurements yet cannot meet the requirements for describing precise and complete information of wing shape and venation. Modern morphometrics uses more direct and intuitive way by superimposing some encoded landmarks' coordinates to solve this problem (Rohlf and Slice 1990). After rotation, translation, and scaling, one can aligns those landmarks as well as possible, and see the differences among specimens. The results of least-squares superimposition of sixteen landmarks for totally 297 wing specimens in six *Liriomyza* species clearly show the relative variations for each landmark among different samples (Fig. 4). Among the selected sixteen landmarks, landmark #2, #3, and #6 present larger variability which have larger ellipse areas of two standard deviations as shown in Fig. 4. Otherwise, landmarks #14 and

#15 present special directional variability that revealed by the flatter ellipses. This indicates that there have special variations happened on the outer cross vein (landmarks #14 and #15 are on the both ends of outer cross vein); and 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 appeared mostly along the longitudinal vein of M_{1+2} and M_{3+4} . Although the landmarks #2, #3, and #6 on wing margin have large variability, the result areas of rounder ellipses of two standard deviations suggest their differences were probably caused by some kinds of random and non-directional changes.

On the other hand, landmark-based geometric morphometrics has recently developed the theories and methods to describe, analyze, and interpret biological shapes and shape variations in more detail (Rohlf and Marcus 1993). Using a special interpolation function of “thin-plate spline” (Bookstein 1989, 1991), we can compute the coordinate transformation between corresponding landmarks on two objects, and reveal the shape differences by D’Acry Thompson’s transformation grids (Thompson 1917). In this study, the thin-plate spline relative warp analysis was computed to summarize the wing variations among 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 the different shape changes were mainly happened on the central area of wing, especially on the location of cross veins (r-m and m-m cross veins) (Fig. 5). And by superimposing the minimum spanning tree onto the scatterplot of 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 *L. trifolii*, *L. brassicae*, and *L. sativae* have resembling overall wing shapes, while *L. chinensis* has relatively different shape that is on the far terminal of this tree. The first and second relative warps account for 57.6 and 30.2% of the total wing variations, respectively.

One-way MANOVA were then performed by using “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 varied significantly among different species. The multiple comparison results of both affine and non-affine components show except within *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*, $p=0.18$, significant unplanned comparisons based on Hotelling’s T^2 values), all pairs between two species have significant shape differences.

The thin-plate-spline-based analysis is a precise tool for exploring shape variation; 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). The complete wing shape information can therefore be compared and analyzed in this study, so it worth further development in application of identifying species, especially when using in detecting minor shape differences between closely related species.

Male genitalia:

Male genitalia is probably the most important taxonomic character in Agromyzidae since Nowakowski (1962), and then it was generally adopted as a major character by most of the leading specialists, such as Spencer (1990), Sasakawa (1963), Griffiths (1974), and Tschirnhaus (1971). Nevertheless, most

of these characters are presented by means of drawings, only few photos (Tschirnhaus 1981) can be found in the literature. Genitalia drawings are somewhat difficult to follow, different authors used different ways to express the genital characters, and the correct species identification is always strongly relied on the quality, precision and details of the drawings. Spencer (1992) has mentioned that the Sasakawa's drawings of genitalia are of the highest quality, and without question the best provided by any specialist on this family. This also implies that he considered the quality of drawings do matter in identifying agromyzid species. However, those genitalia drawings are more or less personalized and somewhat difficult to compare with the real dissected objects, especially for a beginner. For example, some authors use the outline drawings to represent the overall shapes; some prefer dotted drawings to emphasize the different areas or various degree of sclerotization of those hard parts on genitalia. In this study, I carefully dissected the male genitalia and cleaned them using distilled water and alcohol then photographed them. I suppose that using photography of larger focal depth can not only overcome those defects of using traditional photos but also avoid misjudgement by reading from the descriptions or drawings. The terminology used here mainly follows that of Sasakawa (1961) and Spencer (1973).

In the broad sense, male genitalia basically include genital arch (which is composed of epandrium, two surstyli, hypandrium and two cercus), phallus, phallapodeme, and sperm pump. Epandrium is actually the ninth abdominal tergite and frequently with distinctive shape, but it's hard to conclude those shape differences have any discriminative value on which taxonomical level. Same situation also happened when applying surstylus, hypandrium, cerci, and phallapodeme for taxonomical or identification purpose.

Contrarily, the phallus (or aedeagus) of Agromyzidae in most species is so highly evolved and characteristic that Spencer and Steyskal (1986) have mentioned the illustrations in side, ventral, or both views will suffice to permit immediate identification even in species level. The phallus consists of four sections: basiphallus, mesophallus, hypophallus, and distiphallus. According to the photomicrographic results in Fig.6 and Fig. 7, we can see that the distiphallus has the most conspicuous shape, and this is probably the most useful character in identifying *Liriomyza* species. But the problem is when we treated on those closely related species, like the species mentioned in this paper, the morphology of distiphallus is still easily confused without careful examination. There are two pairs of species in my studies which have similar shapes in phallus. *Liriomyza bryoniae* and *L. huidobrensis* is one of the resembling pairs (Fig. 6 A, B and Fig. 7 A, B) which have resembling distiphallus, especially from lateral view (a and a' in Fig. 6). But careful examination will reveal that the ventral views of distiphallus are actually different, where *L. bryoniae* has more rounded bowl-shaped distiphallus when compare with *L. huidobrensis* (a and a' in Fig. 7). In addition, the mesophallus of these two species have different sclerotization areas (b and b' in Fig. 7), but they are obscure without properly preparing samples. Another pair of *L. trifolii* and *L. sativae* is much difficult to be separated, the differences of these two species mainly appear on the distiphallus (Fig. 7 c, c' and Fig. 6 b, b'). However, the differences between these two species are quite hard to describe in writing, and only I can see is the different sclerotizing degree and minor shape changes on distiphallus. In this case, the lateral views (b and b' in Fig. 6) seem much easier to use in separating these two species.

Morphological methods are the earliest and most intuitive way to

recognize and differentiate different living organisms, and are also the foundation on which most taxonomic systems are based. However, new techniques, such as the molecular methods, developed quickly during these years, and gradually substitute those traditional morphological methods that have long been considered subjective. Fortunately, more advanced morphological methods and instruments are now available for re-evaluating those traditional characters used in taxonomy; and even more, to explore new morphological characters. In this article, morphological characters of *Liriomyza* species are examined and evaluated; the purpose is under the consideration of using morphological approaches in quarantine examination for a rapid and more accurate species judgment. Using the electronmicrography and computer-aided image enhancement system, we can have image photo pictures of better quality to assist identification; using modern morphometric analysis, we can precisely locate and quantify differences of wing shapes and wing venations. In conclusion, there is no single character whatever can conveniently and successfully differentiate every *Liriomyza* species, even in genitalia characters, without careful comparisons. The reason is partially caused by the highly homogenous morphology within this group. Nevertheless, the highly similar morphology in *Liriomyza* pest group doesn't match its diversified ecology; different species with different host preferences, parasite complex, and pesticide resistances do deeply affect the quarantine and control policies. And the preliminary species discrimination is only the first basic step to assist the ongoing related studies to look inside the problems.

五、計畫成果自評

潛蠅科昆蟲種類繁多，傳統分類學描述

及記錄的工作並非短時間內可以完備，目前雖已初步建立親緣關係，然常態與持續的進行採集、標本檢查與分析有其絕對的必要性，我們無法排除在更詳盡種類研究後系統關係更動的可能性。本研究在形態與鑑別方面的研究，在應用實務上將來可直接用在解決害蟲防治上區辨近似種的問題。在幾何形態測量學之開發應用方面，本研究已針對傳統形態部份將成果直接在應用層次上獲得初步的實踐。然而，關於形態測量學之理論及方法；自我評估對此新領域的基礎開發及研究上仍嫌不足，對其與系統學之配合研究方面離完整建立仍有一段距離；且在與進化推論之關連上仍未完備；不管在理論與實用層面將來應嘗試持續與數學、統計或其他生物學領域等跨學門之國內外學者作更密切的合作。

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附圖

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*.

Fig. 2. Arrangements of thoracic microsetae of six *Liriomyza* species. A and B indicate the location of microsetae on 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 μ m.

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 μ m.

Fig. 4. Result 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 were presented for each landmark. Arrows indicate the location of landmarks (#14 & #15) on outer cross vein that have directional variation.

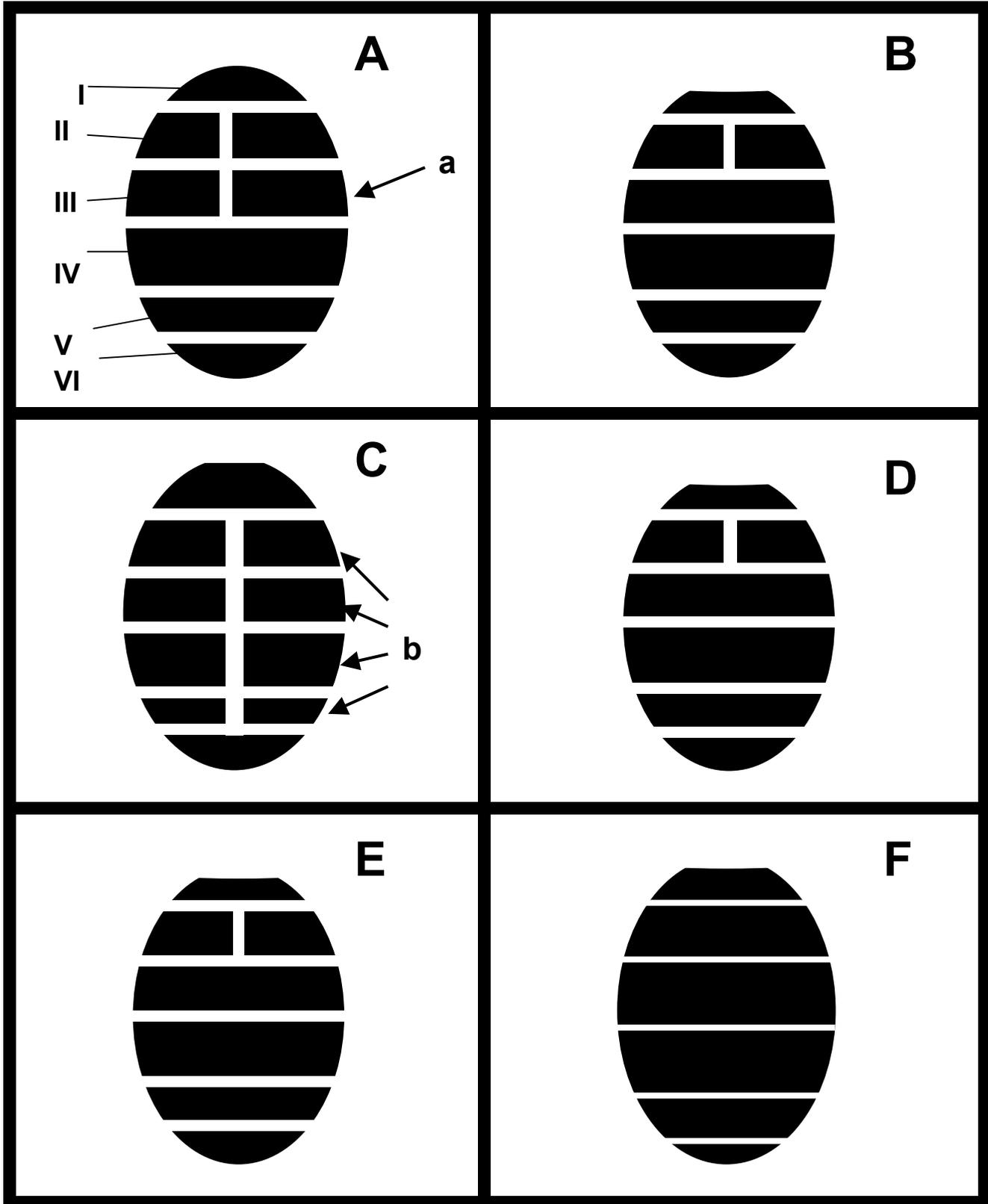
Fig. 5. Minimal spanning tree was superimposed onto the scatterplot of the second relative warp (RW2) against the first relative warp (RW1) of wing shapes of six *Liriomyza* species. Shape changes of each species were represented by deformation grids. The first and second axes account for 57.6 and 30.2%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

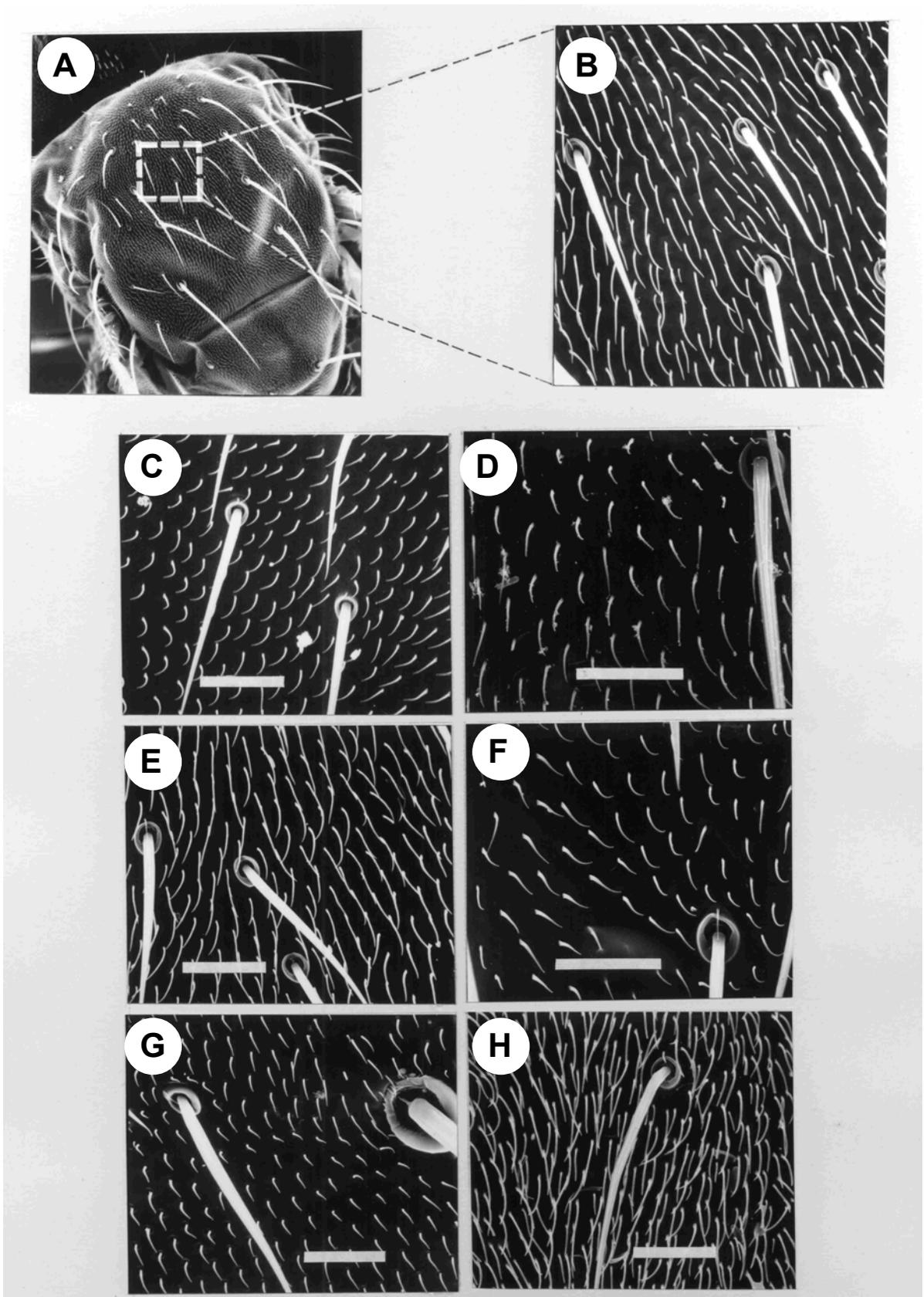
Fig. 6. Photo plates of phallus in six *Liriomyza* species, lateral view. A, *L. bryoniae*; B, *L. huidobrensis*; C, *L. trifolii*; D, *L. sativae*; E, *L. brassicae*; F, *L. chinensis*. a, a', b, b', c, and

d indicate the location of distiphallus. Scale bar = 0.1 mm.

Fig. 7. Photo plates of phallus in six *Liriomyza* species, ventral view. A, *L. bryoniae*; B, *L. huidobrensis*; C, *L. trifolii*; D, *L. sativae*; E, *L. brassicae*; F, *L. chinensis*; a, a', c, c', d, and e indicate the location of distiphallus; b and b' indicate the location of mesophallus. Scale bar = 0.1 mm.

Fig. 1





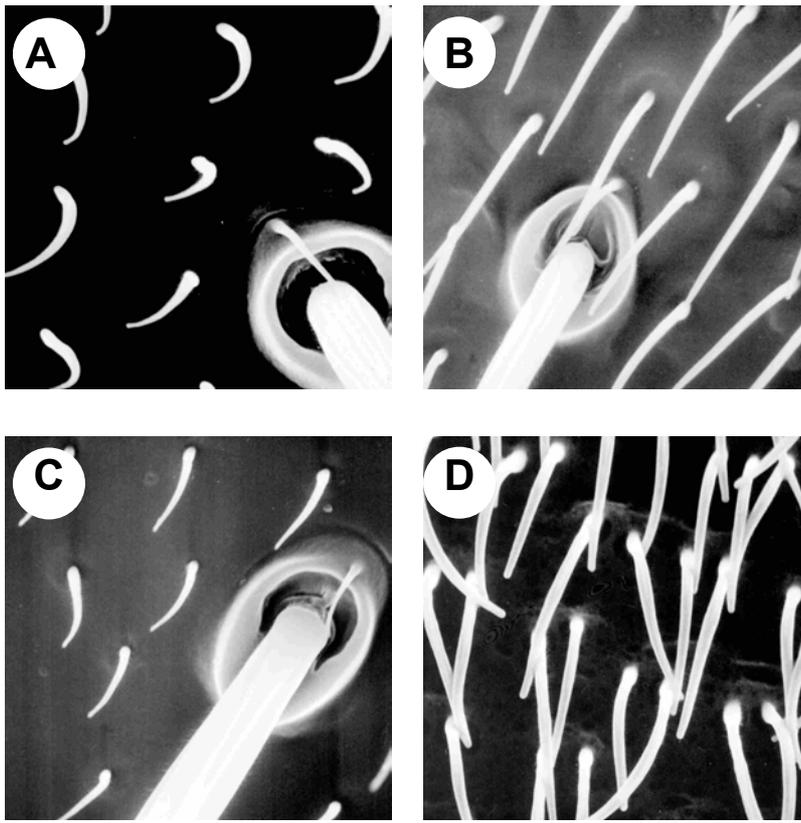
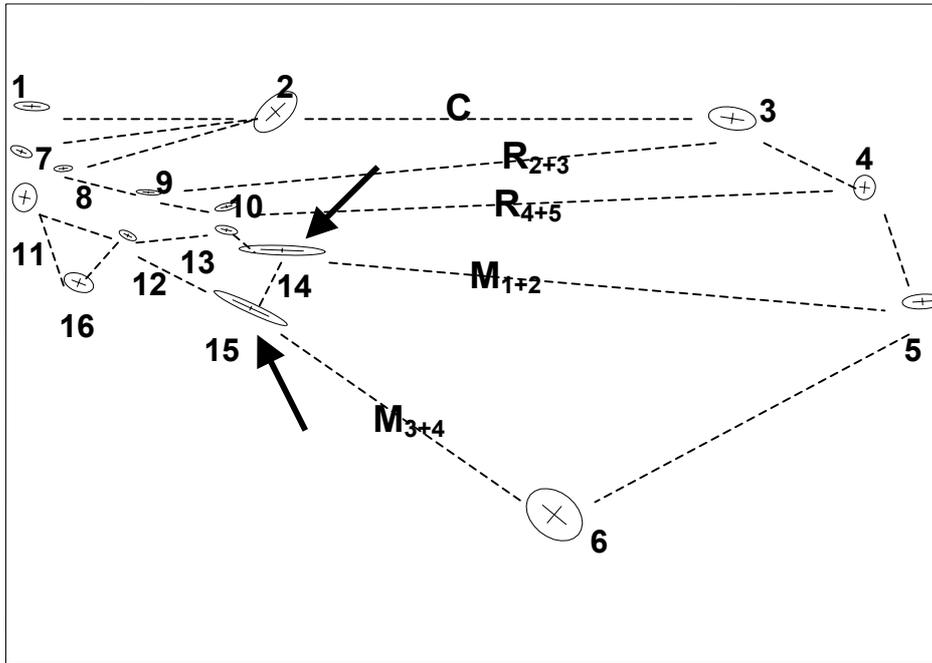


Fig.4



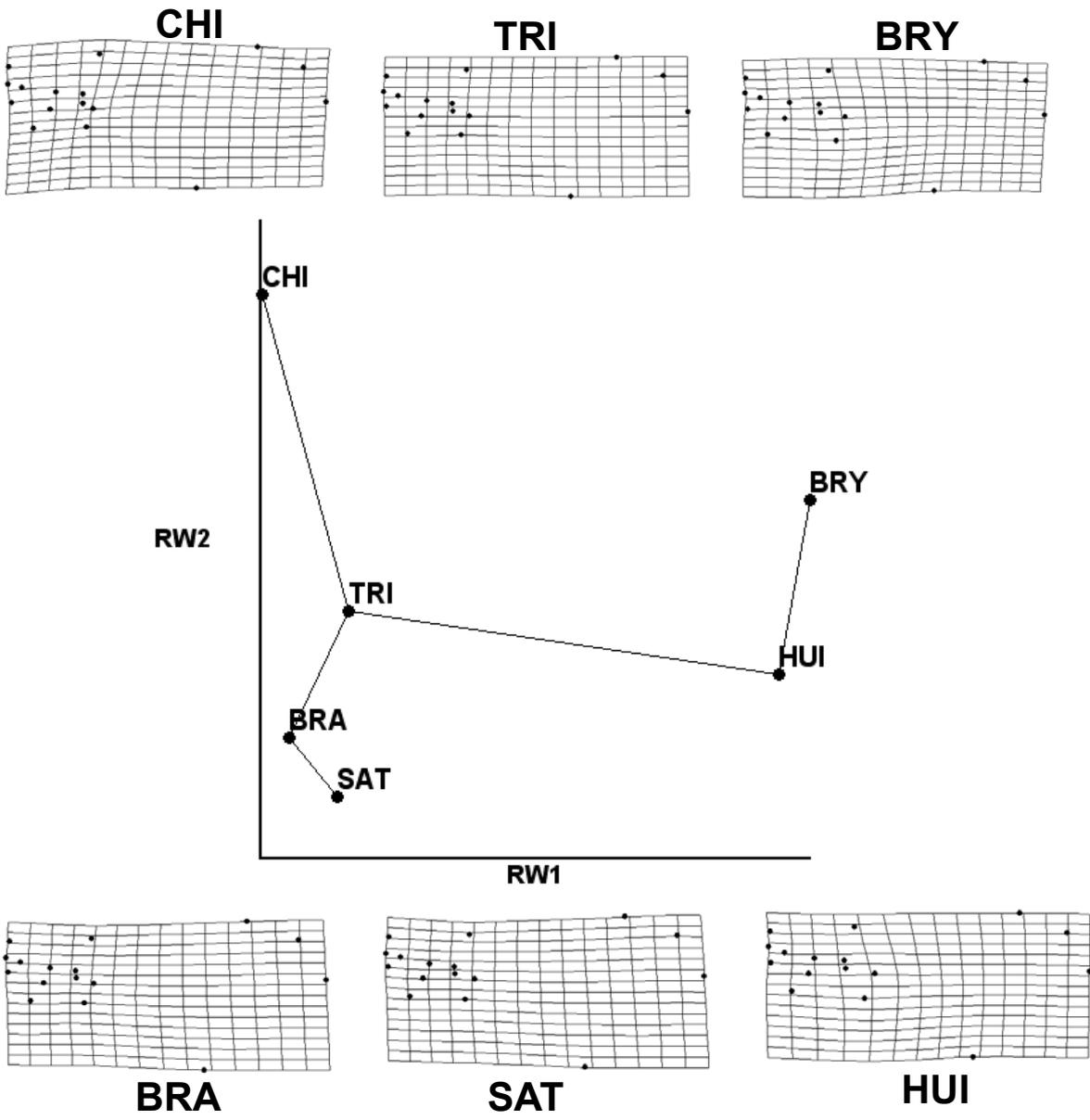


Fig. 6

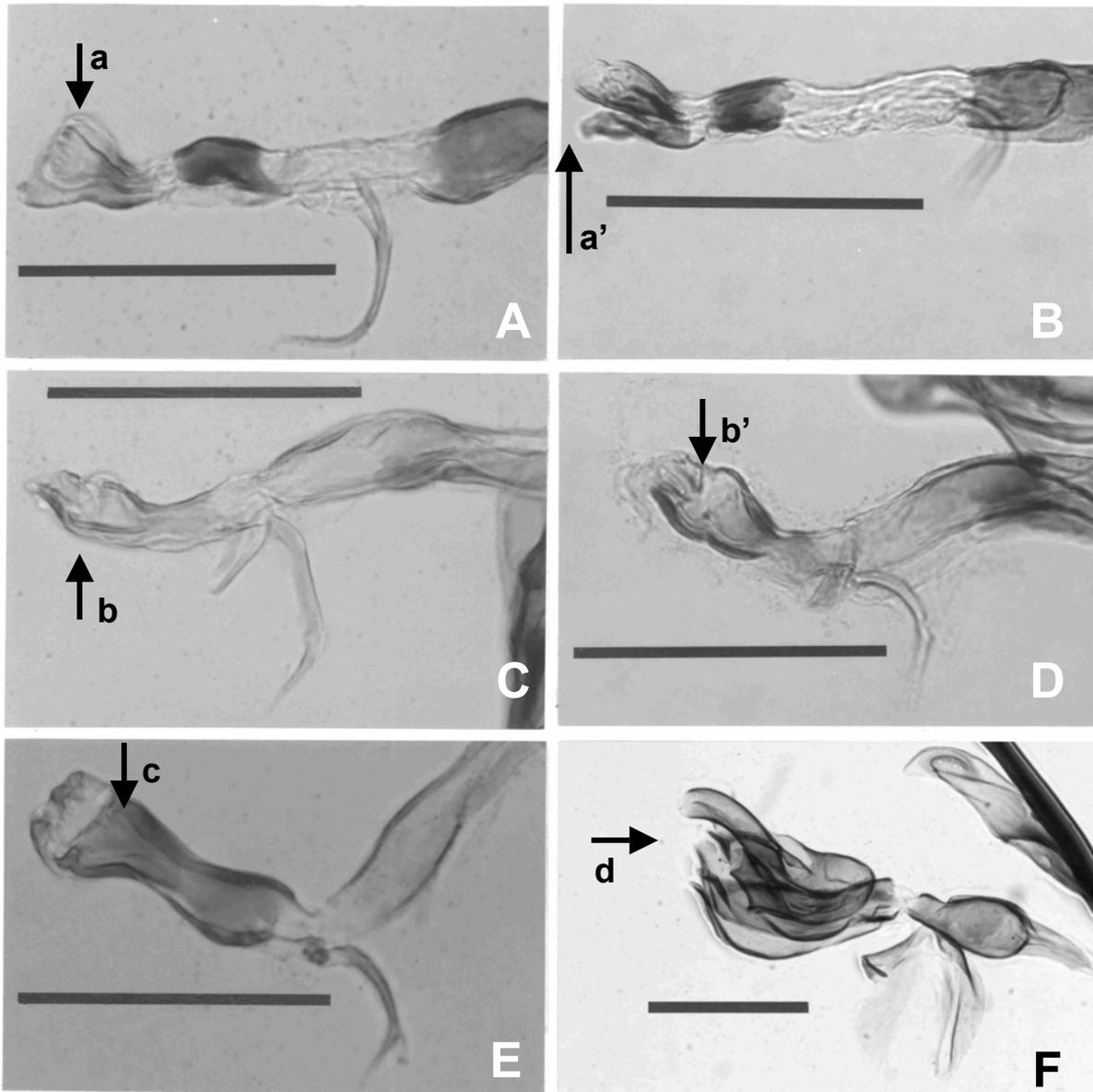


Fig.7

