SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Relative Warp Analysis to Study Morphological Variations in the Genital Capsule of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

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Neotropical Entomology 31(2): 217-224 (2002)

Análise das Deformações Relativas no Estudo da Variação Morfológica na Cápsula Genital de *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

RESUMO - A influência dos hospedeiros sobre a morfologia da cápsula genital masculina de *Trichogramma pretiosum* Riley foi estudada por meio das análises de morfometria geométrica. Foram utilizados espécimes de *T. pretiosum* obtidos de 10 espécies de hospedeiros da ordem Lepidoptera. Inicialmente, estabeleceram-se os marcos anatômicos e foram obtidas as coordenadas cartesianas (x e y) de cada marco para as cápsulas genitais de todos os espécimes. Os marcos anatômicos transformados em coordenadas cartesianas foram analisados pelo método de superposição ortogonal pelos quadrados mínimos. Obteve-se uma configuração de consenso da cápsula genital para todos os espécimes e, em seguida, determinaram-se as deformações parciais e relativas. Quinze marcos geraram 24 deformações relativas para cada espécime. As ordenações das deformações relativas ($\alpha = 0$), usando os componentes de forma, separaram *T. pretiosum* em quatro grupos segundo o hospedeiro (1 – *Alabama argillacea* (Hueb.), *Helicoverpa zea* (Bod.) e *Diatraea saccharalis* (Fabr.); 2 – *Danaus plexippus erippus* (Cramer), *Hamadryas feronia* (Linn.) e *Spodoptera frugiperda* (J.E. Smith); 3 – *Anticarsia gemmatalis* Hueb., *Bonagota cranaodes* Meirick e *Diaphania* sp. e 4 - *Anagasta kuehniella* (Zeller). Os dois primeiros eixos das deformações relativas explicaram 83,0% da variação total da forma dos espécimes. As principais diferenças na forma ocorreram na região anterior da extensão posterior da lâmina dorsal e da cápsula genital. Os resultados obtidos são iniciais e exploratórios, demonstrando que sutis diferenças na forma das cápsulas genitais masculinas de *T. pretiosum* podem ser detectadas por meio das análises de morfometria geométrica.

PALAVRAS-CHAVE: Insecta, morfometria geométrica, parasitóides de ovos.

ABSTRACT – The influence of the host on the morphology of the male genital capsule of *Trichogramma pretiosum* Riley was studied by geometric morphometric analysis. Specimens of the parasitoid obtained from 10 different Lepidoptera host species were used. Initially, landmarks were defined and Cartesian coordinates (x and y) for each landmark of the genital capsule of all specimens were obtained. The anatomic landmarks transformed to Cartesian coordinates were then analyzed by generalized orthogonal least-square procedure. Consensus configuration of the genital capsule for all specimens was obtained and partial and relative warps were determined. Fifteen landmarks generated 24 relative warps for each specimen. The ordination of the relative warps ($\alpha = 0$), using shape components, separated *T. pretiosum* into four different hosts groups: (1 – *Alabama argillacea* (Hueb.), *Helicoverpa zea* (Bod.) and *Diatraea saccharalis* (Fabr.); 2 – *Danaus plexippus erippus* (Cramer), *Hamadryas feronia* (Linn.) and *Spodoptera frugiperda* (J.E. Smith); 3 – *Anticarsia gemmatalis* Hueb., *Bonagota cranaodes* Meirick and *Diaphania* sp. and 4 - *Anagasta kuehniella* (Zeller). The two first axes of the relative warps explained 83.0% of the total variation of the genital capsule shape of the specimens. The main difference was at the anterior region of the posterior extension of the dorsal lamina of the genital capsule. Although results obtained were initial and exploratory they have demonstrated that subtle shape differences in the male genital capsule can be detected by geometric morphometric analysis.

KEY WORDS: Insecta, geometric morphometrics, egg parasitoids.

Trichogramma pretiosum Riley, a parasitoid frequently used in biological control programs, has a wide distribution in the New World where it has been associated to 240 different hosts (Pinto 1998). This species presents low genetic variability and apparent morphological uniformity. Intraspecific variation, however, has been observed mainly in the male genitalia, which is an important parameter in the taxonomy of the genus *Trichogramma*. Studies carried out by Pinto *et al*. (1989) have shown that several characteristics of *T. pretiosum* were plastic and liable to ecophenotypic variation.

Studies on the host influence on the morphology of this parasitoid are scarce. It is known, however, that the parasitized species affects the size of the *Trichogramma* (Marston & Ertle 1973, Kazmer & Luck 1990, Bai *et al*. 1992, Liu *et al*. 1994). Most of the morphometric studies on this genus were based on linear measurements, which are certainly influenced by the size of the individuals sampled. Nevertheless, information about variations in the shape of the morphological structures on *Trichogramma* is not available.

The use of geometric morphometry on the study of shape has caused a revolution on the biometric analysis (Rohlf & Marcus 1993, Bookstein 1996), promoting advances on the knowledge and utilization of this technique in the early 90's (Monteiro & Reis 1999). This method was developed to analyze the differences of shape among organisms based on anatomic landmarks defined by Cartesian coordinates (x and y). These coordinates are compared between specimens after removing the effects of size, position, and orientation, allowing to singly evaluating the differences in shape. Thus, it is possible to say that the shape of the object to be studied is contained within geometric properties that are not altered with changes in scale, translation and rotation (Rohlf 1996). These procedures are described in details in Rohlf & Bookstein (1990), Rohlf (1998a) and Monteiro & Reis (1999).

T. pretiosum presents morphological variation in the genital capsule that may be related to the hosts. The objective of this research was to carry out an exploratory study aiming to detect variations on the shape of the genital capsule of this species using geometric morphometry by means of Relative Warp Analysis.

Material and Methods

Material Examined. The specimens used were obtained from the *Trichogramma* microscope slide collection of the Escola Superior de Agricultura Luiz de Queiroz – ESALQ, Piracicaba county, State of São Paulo, Brazil. Specimens of *T. pretiosum* from 10 different species of the order Lepidoptera were used (Table 1). The microscope slides were selected by choosing specimens presenting intact structures and suitable conditions to be drawn and the number of slides was defined by the availability of material in the collection. Only males were studied since the identification of *Trichogramma* is exclusively based on morphological structures of individuals of this sex.

Data Collection. Genital capsules of *T. pretiosum* specimens were drawn using a camera lucida (16x magnification) connected to a microscope (400x magnification). The drawings were ordinarily performed with the posterior extremity of the genitalia directed to the bottom margin of the paper and were than digitalized by the use of a scanner. A scale was used to standardize the drawings. Fifteen anatomical landmarks (representing homologous points) were defined in each drawing (Fig.1) in regions that better define the morphological differences observed in the structure. The Cartesian coordinates (x and y) of the landmarks were recorded in each drawing using the Tpsdig program (Rohlf 2000), and the types of anatomical landmarks were based on Bookstein (1991) (Table 2).

Geometric Morphometric Analysis. The Tpssmall program was used to quantify the variation in the shape of the genital capsules (Rohlf 1997). This procedure was adopted because when the variation in shape of a group of specimens is very large, statistical methods based on the approximation of the

Table 1. Hosts, collection sites and number of specimens of *T. pretiosum* used to study morphological variations in the genital capsule of this parasitoid using Relative Warp Analysis.

Host insects (Lepidoptera)	Collection sites (county - state)	# of specimens
A. argillacea (Noctuidae)	Juarez Távora-PB[3]	5
A. kuehniella[1] (Pyralidae)	Piracicaba-SP	14
A. gemmatalis (Noctuidae)	Curitiba-PR	6
B. cranaodes (Tortricidae)	Vacaria-RS	8
D. plexippus erippus[2] (Nymphalidae)	Piracicaba-SP	5
Diaphania sp. (Pyralidae)	Viçosa-MG	3
D. saccharalis (Pyralidae)	Rio Brilhante-MS	10
H. feronia[2] (Nymphalidae)	Piracicaba-SP	3
H. zea (Noctuidae)	Lavras-MG	12
S. frugiperda (Noctuidae)	Piracicaba-SP	6

[1] Laboratory rearing - with more than one year of multiplication of the parasitoids
[2] First generation of *T. pretiosum* on the alternative host
[3] PB = Paraíba; SP = São Paulo; PR = Paraná; RS = Rio Grande do Sul; MG = Minas Gerais; MS = Mato Grosso do Sul

tangential space cannot be used.

The configuration of the anatomical landmarks were superimposed by the least-squares method that transforms a configuration of landmarks, superimposing it on a configuration of reference (consensus) translating, scaling and rotating one of them so that the sum of squares of the distances between the corresponding points among configurations would be the least possible (Monteiro & Reis 1999). The configuration of references of the specimens by host were combined to analyze only the differences between the means.

The warps, represented by the thin-plate splines function, were determined and decomposed in shape descriptors which may have uniform components that describe stretching, compression or scission (global variation) and localized components corresponding to changes that occur at specific regions. The principal warps are the eigenvectors of the matrix of the deformation energy and each one describes a possible change in the shape applicable to the main configuration.

The superimposed configurations were then projected on the principal warps describing the differences in shape as deviations of the main configuration (Bookstein 1989, Rohlf 1993). The projections or scores generated indicated how much of each principal warp was needed to accomplish the deformations. The scores, also called partial warps, described each genital capsule of the specimens as a linear combination between principal warps and the standardized coordinates x and y for each anatomic landmark. These scores can be used in multivariate analysis (Rohlf 1993, Rohlf *et al*. 1996).

The relative warps, which are the main components of

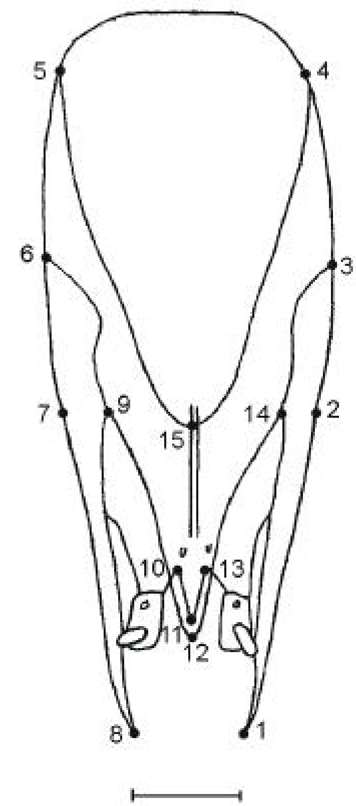

Figure 1. Location of anatomical landmarks in the male genital capsule of *T. pretiosum*.

Table 2. Descriptions and types of anatomical landmarks of the male genital capsule of *T. pretiosum*.

Anatomical landmarks	Descriptions[1]	Type
1	Apex of the right paramerer	II
2	Perpendicular projection, in the right lateral of the genital capsule, of the junction of the posterior extension of the dorsal lamina with the right paramerer	I
3	Insertion of the dorsal lamina in the right lateral of the genital capsule	I
4	Right anterior extremity of the dorsal opening	II
5	Left anterior extremity of the dorsal opening	II
6	Insertion of the dorsal lamina in the left lateral of the genital capsule	I
7	Perpendicular projection, in the left lateral of the genital capsule, of the junction of the posterior extension of the dorsal lamina with the left paramerer	I
8	Apex of the left paramerer	II
9	Junction of the posterior extension of the dorsal lamina with the left paramerer	I
10	Left base of the intervolselar process	II
11	Apex of the intervolselar process	II
12	Apex of the dorsal lamina	II
13	Right base of the intervolselar process	II
14	Junction of the posterior extension of the dorsal lamina with the right paramerer	I
15	Posterior extremity of the dorsal opening	I

[1]See Pinto (1998) for definition of morphological terms.

the matrix that combine scores of partial warps and uniform components, were obtained using the matrix of the scores of the partial warps. *T. pretiosum* ordination graphs and grids of the warps associated to each computed axis were built. In order to have all the partial warps is the same scale of variation, values of $\alpha = 0$ were established in the computation of the scores of partial warps. This procedure is the most suitable for exploratory and taxonomic studies (Rohlf 1993) since the α values displace the axis of higher variation of shape to regions with bigger or smaller location (Monteiro & Reis 1999).

The analyses of superimposing and relative warps were performed using the Tpsrelw program (Rohlf 1998b). All programs of the "TPS" series used in this research work are *freeware* (http://life.bio.sunysb.edu/morph).

Results

Data provided by generalized orthogonal least-square superimposition procedure of *T. pretiosum* genital capsules have shown variation in shape due to differences in the samples. This can be observed by the dispersion of the ponts related to the specimens from 10 different hosts (clear circles) as compared to the average shape (black circles). The largest variation in shape occurred at the anterior region of the genital capsule (Fig. 2).

The 15 anatomic landmarks generated 24 relative warps for each *T. pretiosum* sample. Analysis of relative warps, using shape components, ordered groups of *T. pretiosum* (Fig. 3). The first two axes of relative warps explained 64.3% and 18.7%, i.e., 83.0% of the total variation of the genital capsules shape of the specimens, dismissing the use of other axes.

Trichogramma pretiosum was ordered in relation to the total differences (global and localized) in the shape of the genital capsule (Fig. 3). The grids of hypothetical warps indicate that the parasitoids obtained from hosts located on a given position in the axes may present warps similar to those of the nearest hypothetical grid (Fig. 3 A – D).

Four groups of *T. pretiosum* were determined (Fig. 4). One group was obtained from *Alabama argillacea* (Hueb.), *Diatraea saccharalis* (Fabr.) and *Helicoverpa zea* (Bod.) that presented more dilated relative warp grids at the posterior extension of the dorsal lamina (Fig. 5, landmarks 2 and 7) and at the anterior region of the genital capsule (Fig. 5, landmarks 3 to 6), indicating that the specimens of this group have genital capsules relatively wider. Other group aggregated *T. pretiosum* specimens obtained from *Danaus plexippus erippus* (Cramer), *Hamadryas feronia* (Linn.) and *Spodoptera frugiperda* (J.E. Smith), with narrower grids, indicating that these specimens present narrower genital capsules. *T. pretiosum* obtained from *Anticarsia gemmatalis* Hueb., 1818, *Bonagota cranaodes* Meirick and *Diaphania* sp. comprised a little warped grid group, similar to each other, indicating that these specimens present genital capsule in an intermediate position in relation to the other groups. The specimens of *T. pretiosum* obtained from *Anagasta kuehniella* (Zeller) presented a grid of relative warp with a narrowing at the anterior region (Fig. 5, landmarks 2 to 7) and dilation at the posterior region (Fig. 5, landmarks 1 and 8).

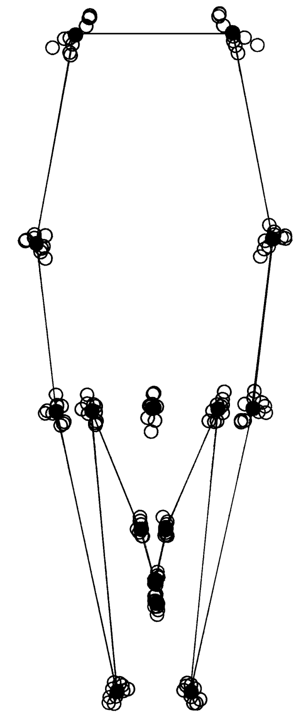

Figure 2. Configuration of anatomical landmarks of specimens of *T. pretiosum* superimposed by least-square procedure. The lines connect landmarks of the mean configuration (black circles) and the variation in shape can be observed around each landmark (clear circles).

Based on the grids of relative warps of *T. pretiosum* it was found that the differences in shape occurred mainly at the anterior region of the posterior extension of the dorsal lamina, at the insertion of the dorsal lamina and at the anterior region of the genital capsule.

Discussion

The relative warps detected the association between *T. pretiosum* and each one of the different hosts, concerning shape of the genital capsule. The host influence on the biology of theses parasitoids is widely discussed in the literature (Marston & Ertle 1973, Kazmer & Luck 1990 and Corrigan & Laing 1994) and the majority of papers discuss the influence of the host species on the size of the parasitoid (e.g. Grenier *et al*. 2001). However, morphological changes caused by the hosts have not been reported.

Results obtained in the present research work do not allow inferences of the size of the host egg on the variation in shape of the genital capsule. However, information from literature indicates that these alterations can occur. Specimens of *T. pretiosum* obtained from bigger size and volume eggs [example: *D. saccharalis* (size: 1136.0 µm x

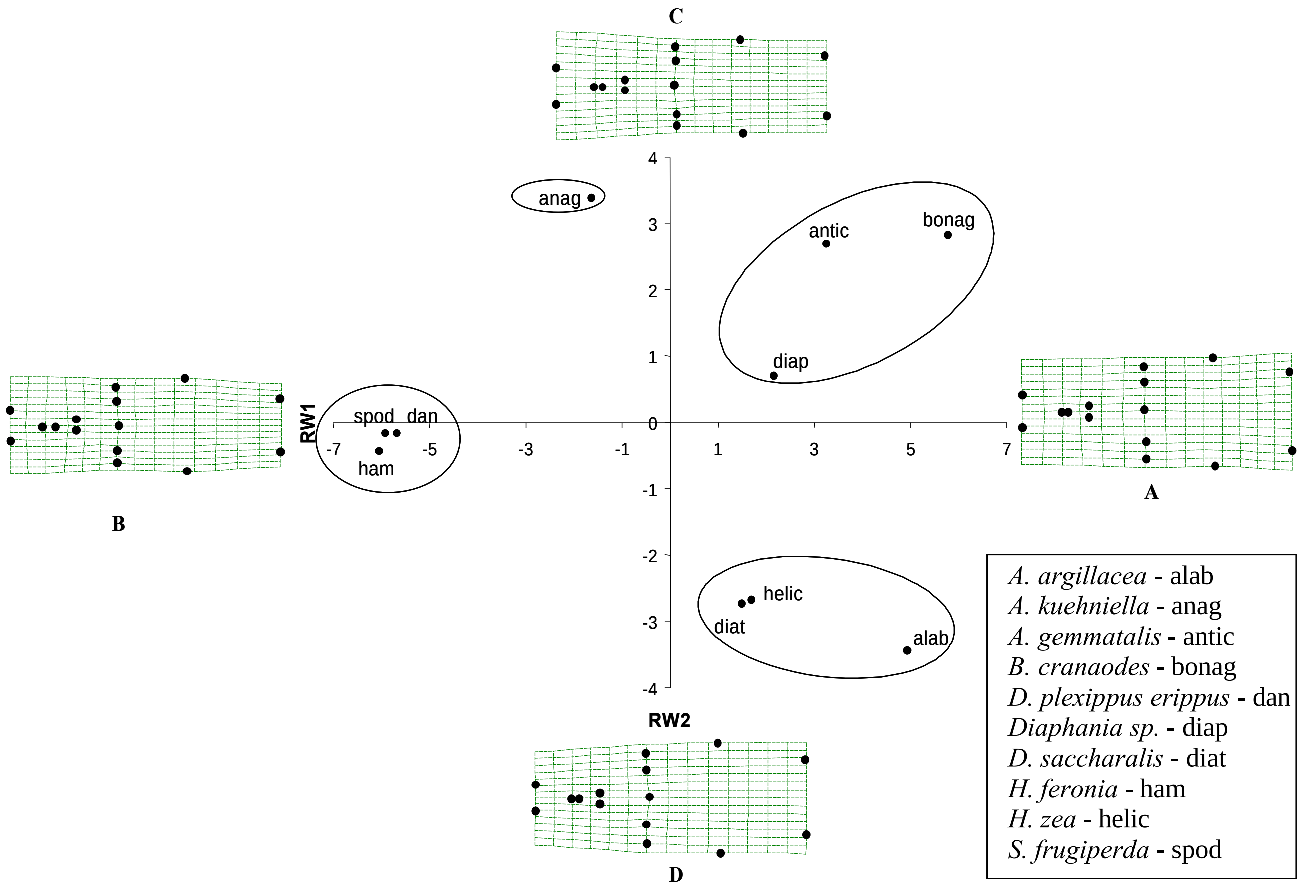

Figure 3. Relative Warps (RW) for values of α= 0 of *T. pretiosum* specimens obtained from 10 different Lepidoptera hosts. A-variation in the shape of the genital capsule foreseen as positive deviations of the mean in the axis of relative warps 1. B-variation in the shape of the genital capsule foreseen as negative deviations of the mean in the axis of relative warps1. C- variation in the shape of the genital capsule foreseen as positive deviations of the mean in the axis of the relative warps 2. D-variation in the shape of the genital capsule foreseen as negative deviations of the mean in the axis of relative warps 2. (Genitalia apex to the left; anatomical landmarks see Fig. 1).

826.9 µm) (Consoli 1997)] presented genital capsule relatively larger than those specimens obtained from smaller eggs hosts such as the ones of *A. kuehniella* (520.7 µm x 289.8 µm; 0.023 mm^3), *S. frugiperda* (454.9 µm x 390.2 µm; 0.036 mm^3) and *A. gemmatalis* (628.2 µm x 428.0 µm; 0.089 mm^3)(Consoli 1997) that presented relatively smaller width at the genital capsule.

T. pretiosum presents intra-specific variation, i.e., many morphological characteristics are plastic and liable to ecophenotipic variation (Pinto *et al*. 1989, Pinto & Stouthamer 1994). In this work, however, the reduced number of specimens obtained from each different host did not allow a multivariate analysis correlating intra- and inter-population variations. By means of sequencing the ITS2 region of the rDNA, Ciociola Jr. *et al*. (2001) compared specimens of *T. pretiosum* obtained from different hosts originated from different regions of Brazil and found that small genetic differences exist between populations but the sequencing pattern within the species remained the same.

Several factors, either genetic or possibly due to host effects (size or nutritional value of the egg), may interact causing differences in shape. To identify these factors, however, more specific studies are needed. Further studies aiming this goal should be carried out trying to correlate these causes to environmental and genetic variables. Although results here obtained were preliminary and exploratory they have demonstrated that the geometric morphometry, using Relative Warps Analysis, can become an important tool in taxonomic studies of the genus *Trichogramma*.

Acknowledgments

To Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP for granting the scholarship to the first author (process # 98/01979-9) and to Professor Dr. Leandro Rabello Monteiro (UNEF) for support and suggestions in reviewing the manuscripts.

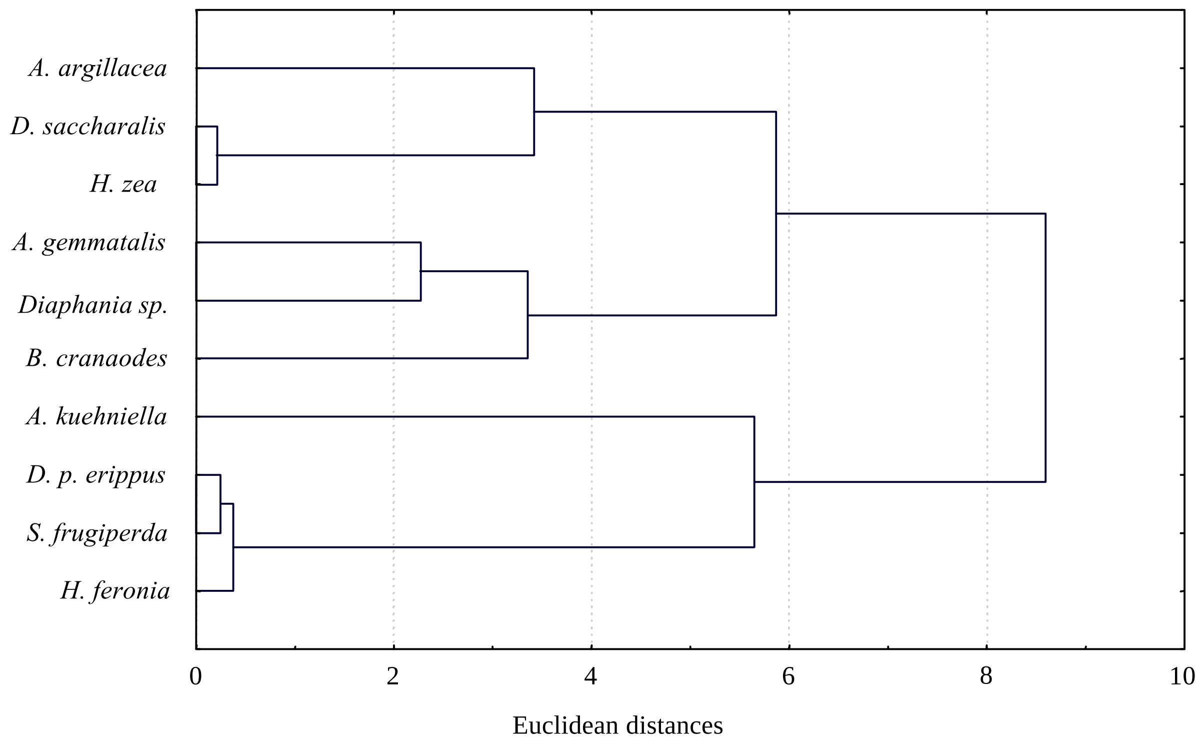

Figure 4. Dendrograph for *T. pretiosum* specimens obtained from 10 different Lepidoptera hosts based on Euclidian distances computed from scores of relative warps ($\alpha = 0$).

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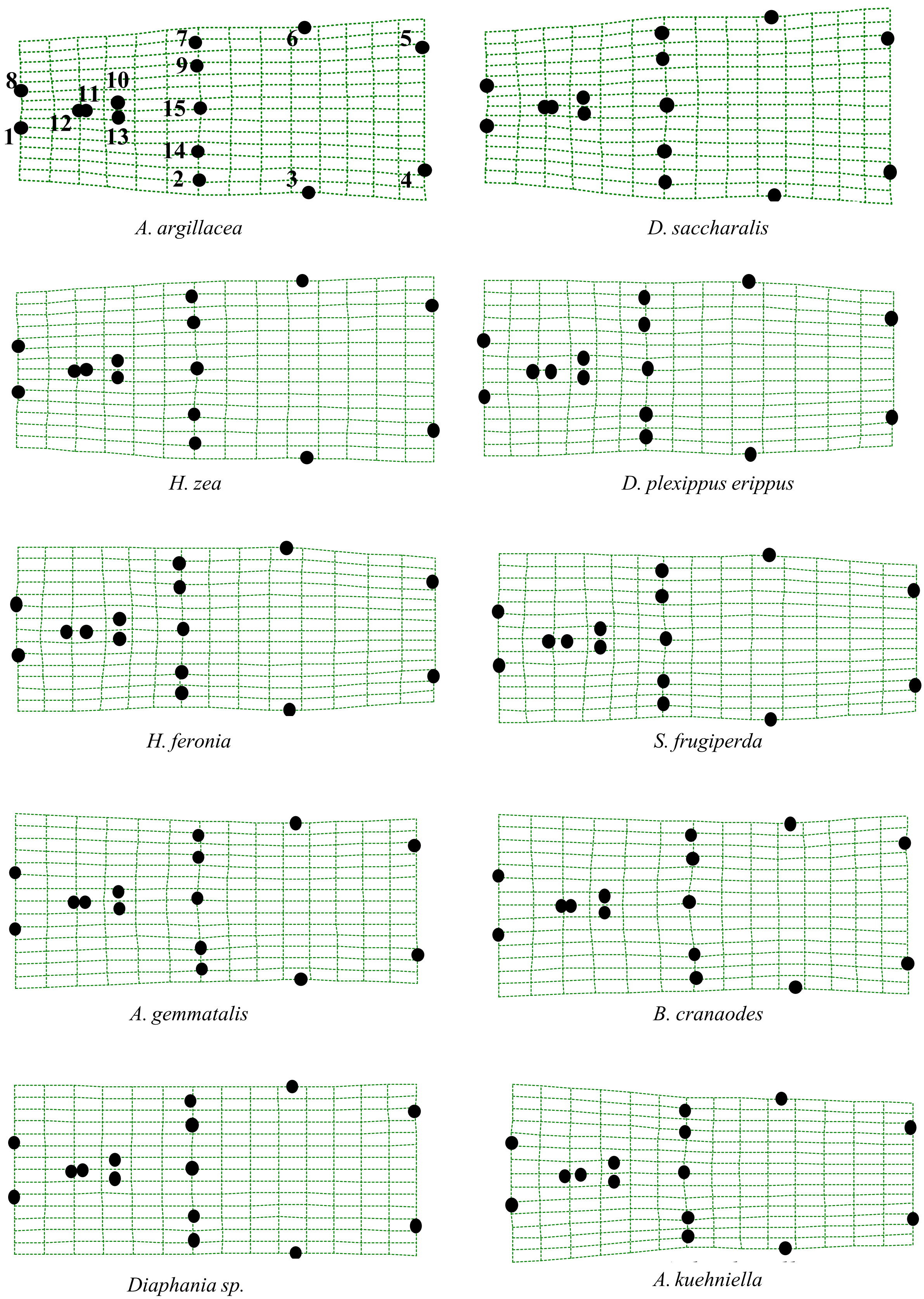

Figure 5. Grids of relative warps (α= 0) with the average shape of the genital capsules of *T. pretiosum* specimens obtained from 10 different Lepidoptera hosts (genitalia apex to the left; anatomical landmarks 1 to 15, see Fig. 1).

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Received 21/08/01. Accepted 10/04/02.