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Revisiting the taxonomy of the genus *Diatraea* Guilding with a focus on *Diatraea tabernella* Dyar, using dichotomous keys, in Panama

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Abstract

Diatraea tabernella Dyar is a key pest of sugarcane in Panama and causes significant crop losses. The objective of this study was to conduct a literature review of the *Diatraea* genus in Panama, focused mainly on *D. tabernella*, complemented with the identification of the most important morphological characteristics to determine the species *D. tabernella*. Reference data were collected from literature about *Diatraea* genus focused mainly in Panama. Adults, larvae and pupae were collected, preserved and later identified in the laboratory. Of the 41 species of the genus *Diatraea* reported in the most recent literature, seven are present in Panama, including *D. tabernella*. The most important morphological characteristics in *D. tabernella* were the presence of lateral lobes of the tegumen in male genitalia (adult), thoracic dorsal mesothelium (larva) and cephalic horns (pupa). The combination of these characteristics allows the identification of the species.

Keywords: Genus, species, taxonomy, morphological characteristics, Stemborer

1. Introduction

Diatraea tabernella is the most important pest in sugarcane in Panama ^[1, 2] causing significant yield losses ^[3]. Like any other lepidoptera stemborer, this species decreases sugar production by causing internal perforations into the sugarcane stalks ^[4-10]. Therefore taxonomic information is essential to formally identify pest species and develop appropriate management strategies. For example, this is of major importance for the success of biological control programs. The objectives of this study is to present a brief review of the taxonomy and description of the genus *Diatraea*, focused mainly on *D. tabernella*, and to identify morphological characteristics using larva, pupa and adult genitalia (male) to identified *D. tabernella* collected from sugarcane fields in Panama.

2. Materials and Methods

Reference data were collected from literature about *Diatraea* genus focused mainly in Panama was used using bibliographic resources from: Web of Science Core Collection and Google Scholar.

Adults, pupae and larvae of *D. tabernella*, were collected from field experiments on specific sampling points during 26 months (2015-2017) in sugarcane fields at Compañía Azucarera La Estrella (CALESA), Natá, Panama (Coordinates: N 08°17.448', W 080°30.402'). The individuals found were preserved in glass vials containing 90% alcohol with a label mentioning the date, and the place of capture.

For the identification of larvae and pupae, we used a stereo microscope (Leica® EZ4 D) with a zoom range from 8x-35x with 10x eyepieces.

For the preparation and identification of the male genitalia we dissolved the insect material in a solution of 10% of potassium hydroxide. The samples were then heated for 10 minutes and left in the laboratory for 4 days at a room temperature of 25 °C. After this operation, materials were washed using 90% alcohol to remove the genitalia from the abdomen tissue. This operation was conducted in a 4-hole porcelain battery to clean the genitalia for 10 minutes per hole. With the help of a media such as Euparal balsam micro-preparation slides of the genitalia were mounted and identified using a microscope (Leica ® DM100) using 10x/0.25 and 40x/0.65 lenses.

Morphological description of *D. tabernella* was based on adults, larvae and pupae of field collected specimen.

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3. Results and Discussion

3.1 Literature review of *D. tabernella* in Panama

The Larvae of this group of stemborers feed on stalks of Poaceae species such as sugarcane (*Saccharum officinarum* L.), corn (*Zea mays* subsp. *mays* L.), rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* (L.) Moench) as well as on other native grasses [11]. Various descriptions of the genus *Diatraea* have been conducted since the subsequent systematic history of the main species of the genus and its economic importance was mentioned in 1828 by the Reverend Lansdown Guilding [12, 13]. The first overall description of *Diatraea* and the related genus was conducted in 1927 by Dyar & Heinrich (22 species and 9 new species described) including the species *Diatraea saccharalis* F. *D. tabernella* Dyar and *Diatraea guatemalensis* Schaus using male and

female genitalia [14]. This work was later revised by the publication of Holloway in 1928 [15] and by Box in 1931 [16], who recognized 48 species, including 10 new species. The group was then verified by Bleszynski in 1969 [17]. The most recent taxonomy revision of the genus was made by Solis and Metz in 2016 [11] and included 41 valid species (Including a checklist with 46 synonyms). This latest publication includes one dichotomous key of the male and female genitalia of the genus *Diatraea* using photos on micro-preparations, while previous descriptions were based on drawings [15, 17].

Chronologically, these lists were generated by Bleszynski (1967) [18], with 55 species, followed by Munroe *et al.* (1995) [19], with 57 species, Nuss *et al.* (2015) [20] with 58 species and Solis and Metz (2016) [11], with 41 species (Table 1).

Table 1: List of species of *Diatraea* generated by Solis and Metz, 2016

No.	Species of <i>Diatraea</i>	No.	Species of <i>Diatraea</i>
1	<i>Diatraea albicrinella</i> Box, 1931	22	<i>Diatraea lentistrialis</i> Hampson, 1919
2	<i>Diatraea andina</i> Box, 1951	23	<i>Diatraea lineolata</i> (Walker, 1856)
3	<i>Diatraea argentina</i> Box, 1951	24	<i>Diatraea lisetta</i> (Dyar, 1909)
4	<i>Diatraea bellifacella</i> Dyar, 1911	25	<i>Diatraea magnifacella</i> Dyar, 1911
5	<i>Diatraea brunnescens</i> Box, 1931	26	<i>Diatraea maronialis</i> Schaus, 1922
6	<i>Diatraea busckella</i> Dyar & Heinrich, 1927	27	<i>Diatraea minimifacella</i> Dyar, 1911
7	<i>Diatraea castrensis</i> Dyar & Heinrich, 1927	28	<i>Diatraea mitteri</i> Solis, 2015
8	<i>Diatraea cayennella</i> Dyar & Heinrich, 1927	29	<i>Diatraea muellerella</i> Dyar & Heinrich, 1927
9	<i>Diatraea centrella</i> (Möschler, 1883)	30	<i>Diatraea myersi</i> Box, 1935
10	<i>Diatraea considerata</i> Heinrich, 1931	31	<i>Diatraea pedibarbata</i> Dyar, 1911
11	<i>Diatraea cramboides</i> (Grote, 1880)	32	<i>Diatraea postlineella</i> Schaus, 1922
12	<i>Diatraea dyari</i> Box, 1930	33	<i>Diatraea ragonoti</i> Box, 1948
13	<i>Diatraea evanescens</i> Dyar, 1917	34	<i>Diatraea rufescens</i> Box, 1931
14	<i>Diatraea fuscilla</i> Schaus, 1922	35	<i>Diatraea saccharalis</i> (Fabricius, 1794)
15	<i>Diatraea gaga</i> Dyar, 1914	36	<i>Diatraea schausella</i> Dyar & Heinrich, 1927
16	<i>Diatraea grandiosella</i> Dyar, 1911	37	<i>Diatraea strigipennella</i> Dyar, 1911
17	<i>Diatraea guatemalensis</i> Schaus, 1922	38	<i>Diatraea suffusella</i> Box, 1931
18	<i>Diatraea impersonatella</i> (Walker, 1863)	39	<i>Diatraea tabernella</i> Dyar, 1911
19	<i>Diatraea indigenella</i> Dyar & Heinrich, 1927	40	<i>Diatraea venosalis</i> (Dyar, 1917)
20	<i>Diatraea instructella</i> Dyar, 1911	41	<i>Diatraea veracruzana</i> Box, 1956
21	<i>Diatraea lativittalis</i> (Dognin, 1910)		

In 1931 and 1935 [13, 16], Harold E. Box already mentioned the major *Diatraea* species present in Panama, namely *Diatraea saccharalis* (F.), *Diatraea lineolata* (Wlfr.), *Diatraea busckella* Dyar & Heinr., highlighting *Diatraea tabernella* Dyar as the most important stalk borer of sugarcane in the country. Following this paper, other studies also highlighted the importance of *D. tabernella* as a key pest of economic incidence in sugarcane in Panama [17, 21, 22]. This species is also mentioned as pest in the region of Central America and Colombia (Cauca Valley), with another close species *D. indigenella* [23-26]. More recently, Solis and Metz (2016) [11] presented an updated list of 7 species present in Panama (Table 2).

Table 2: List of *Diatraea* species in Panama (Solis and Metz, 2016)

No.	Species
1	<i>Diatraea bellifacella</i> Dyar, 1911
2	<i>Diatraea busckella</i> Dyar & Heinrich, 1927
3	<i>Diatraea gaga</i> Dyar, 1914
4	<i>Diatraea lineolata</i> (Walker, 1856)
5	<i>Diatraea lisetta</i> (Dyar, 1909)
6	<i>Diatraea saccharalis</i> (Fabricius, 1794)
7	<i>Diatraea tabernella</i> Dyar, 1911

Within the Crambinae subfamily, *Diatraea* is morphologically defined by a combination of derived

characteristics including a lack of ocelli on the head. The presence of pockets with specialized male scales on the second abdominal segment tufts of hairs on the male posterior tibia, in the male genitalia of basal extensions of the tegumen in some species [27]. Another potentially derived structure in the male genitalia might be the lack of muscle attachments in the lateral lobes of the tegumen [11]. The most important description was made by Holloway *et al.* (1928) [15] in the USA describing in detail the characteristics of all stages of *Diatraea* species, eggs, larvae, pupae and adults that served as a reference for subsequent studies.

They described the species *D. tabernella* as a moth of medium size, variable in size with brownish straw-colored anterior wing; non-aligned veins; two oblique, parallel, dotted outer lines; Discal dot small, black. Regarding the male, the lines are distinct, the posterior wing tinged with brown, while in the female the lines are fade or obliterate, the soil color lighter, more yellowish, while the hind wings are white. In the male, of the posterior tibia has a large tuft of straight lines, curves, blackish hairs. The front face of the head is conical produced with a central tubercle.

Size of the body: Male: 18-28 mm. Female: 25-39 mm. Male genitalia are similar to those of *D. saccharalis* except narrower lateral lobes of the tegumen (much longer than wide), different forms of costal basal costal projection,

weaker gnathos thorns extending for only about one third of its length from the apex (Fig. 1 A). Female genitalia as in *D.*

saccharalis (Fig. 1 B). Abdomen of males with a pair of lateral tufts on the second segment.

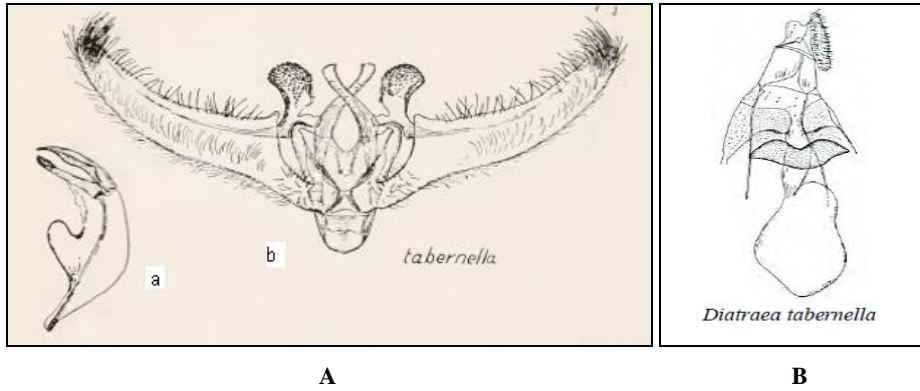


Fig 1 (A-B): description of *D.tabernella* genitalia by Holloway *et al.* 1928; A) male (a. Lateral view, b. Ventral view) and B) female

Later Bleszynski (1969) [17] generated a dichotomous key that considered adult traits and male and female genitalia for *D.*

tabernella (Fig. 2 A-B).

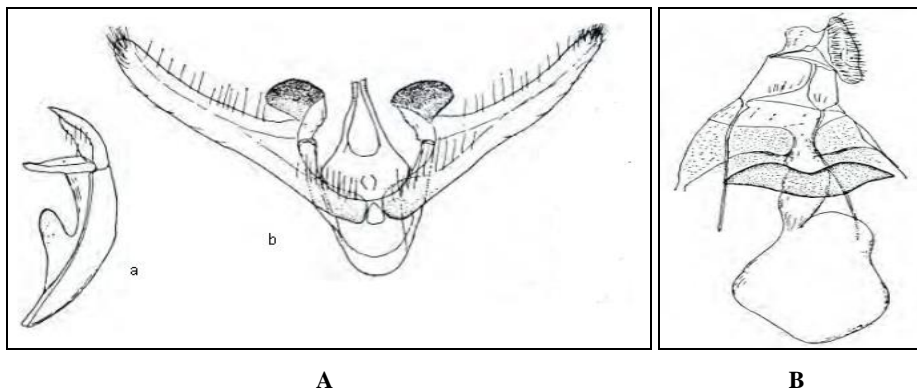


Fig 2(A-B): Description of *D.tabernella* genitalia A) male (a. Lateral view, b. Ventral view) and B) female

Vargas *et al.* (2013) [25] in larvae suggested the use of the meso-thoracic dorsal shield as a means of distinguishing among the species of *Diatraea* given the great difficulties of the separation of *Diatraea* species using immature stages. The pupal stage provides more elements to distinguish species. For example, the cephalic horns in the *D. saccharalis* pupa are pointed at the end (ridge-like) whereas in *D. tabernella* they are rounded as in *D. indigenella* but shorter. Regarding the adult stage, the differences between the species are more pronounced and the posterior tibia of *D. tabernella* have a large clump of blackish hairs absent in *D. saccharalis* [17]. Following the descriptions proposed by Bleszynski (1969), Vargas *et al.* (2013) [25] prepared a key that considers three species found in the Cauca River Valley, Colombia, including *D. saccharalis* and *D. tabernella* (the most important specie in Panama), where they can be

distinguished using male genitalia and, more precisely, the lateral lobes of the tegumen. In *D. saccharalis* tegumen the lateral lobes are larger and more rounded than in *D. indigenella*. Regarding *D. tabernella* the lateral lobes are quite similar to those of *D. saccharalis*, but much narrower and tapered.

The preparation of the genitalia for identification, following the methodology from Clarke (1941) [28] and Robinson (1976) [29], includes the introduction of the female (Fig. 3A) or male (Fig. 3B) body in a bottle with a solution of 10% of potassium hydroxide (KOH). The flask is then placed in a beaker with boiling water. If staining is needed to see structures, the use of a saturated solution of black chlorazol for just a few seconds is required. Then, the genitalia parts can be stored in vials with glycerine if available, or 70% alcohol.



Fig 3(A-B): A (Female); B (Male) Adult of *Diatraea* spp. (José Daniel Salazar (LAICA-DIECA))

3.2 Identification based on *D. tabernella* morphological characteristics.

During the sampling period 174 larvae (94.5%) (20 small, 147

medium and 7 large), 11 pupas (5.9%) and 11 adults were captured (Table 3).

Table 3: The total captures per stage of *D. tabernella*

Stage	Harvest 2015	%	Growth 2015	%	Harvest 2016	%	Growth 2016	%	Harvest 2017	%	Total	%
Larva small (-2 cm)	4	21.05	1	4.55	2	6.67	13	16.25	0	0.00	20	10.81
Larva medium (2-3 cm)	11	57.89	19	86.36	28	93.33	62	77.50	27	79.41	147	79.46
Larva large (+3 cm)	2	10.53	0	0.00	0	0.00	5	6.25	0	0.00	7	3.78
Pupa	2	10.53	2	9.09	0	0.00	0	0.00	7	20.59	11	5.95
Total	19	100.00	22	100.00	30	100.00	80	100.00	34	100.00	185	100.00

Using the samples collected, the morphological characteristics for identification of *D. tabernella* were detailed:

Male genitalia of *D. tabernella* (micropreparations) with lateral lobes of the tegumen, longer than broad (Fig. 4 A. Lateral view - t = tegumen, lt = lateral lobes of the tegumen; Fig. 4 B. Ventral view - v = valvae).

of *D. tabernella* with thoracic dorsal mesothelium that shows Larva a narrow median incision (im= incision medial; tm= Thoracic mesothelial tube) (Fig. 5 A-B)

Pupa of *D. tabernella* with cephalic horns in the pupa are dull and round and short, at the end (ch=cephalic horns) (Fig. 6)

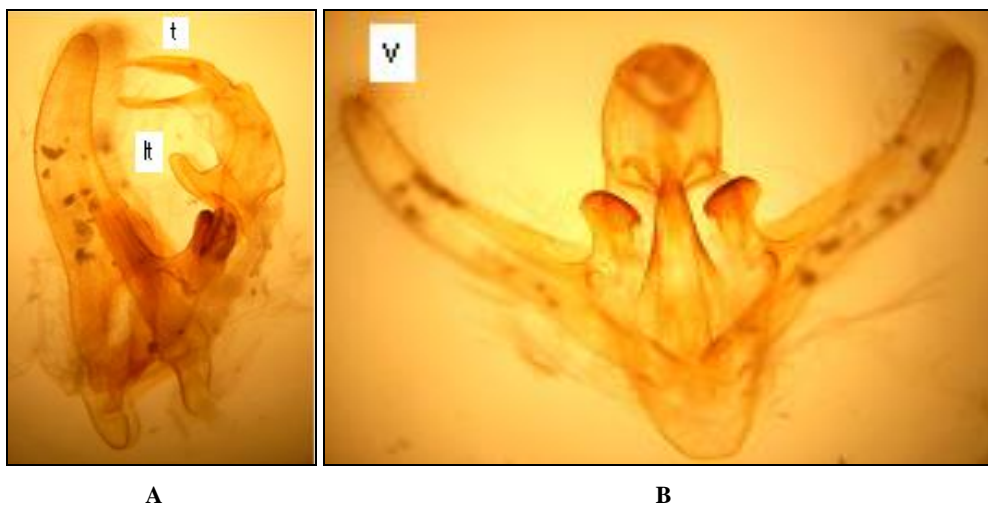


Fig 4(A-B): Male genitalia of *D. tabernella* (micropreparations)

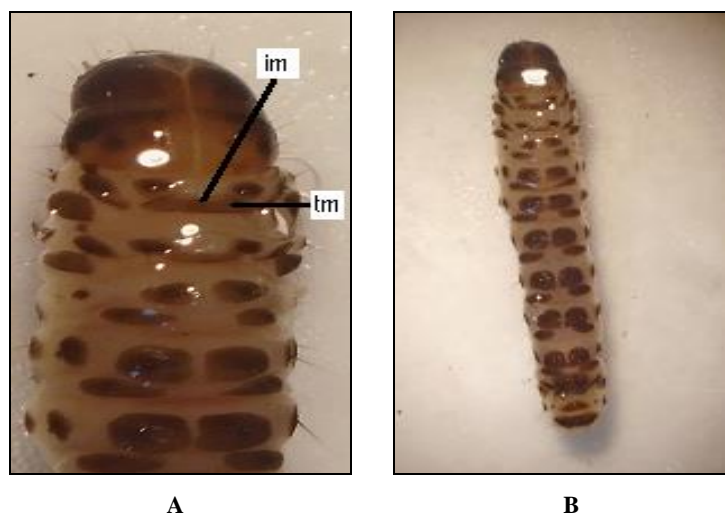


Fig 5(A): Larva of *D. tabernella*



Fig 6: Pupa of *D. tabernella*

4. Conclusions

Of the 41 species of the genus *Diatraea* reported in the most recent literature, seven (17.07%) are present in Panama, with *D. tabernella* being the most important pest in sugarcane plantations. This study allowed to characterize a major species present (*D. tabernella*) in sugarcane fields in Panama using dichotomous keys of adults, larvae and pupae already existing in the literature. The next step will be to work on molecular characterization of the species in Panama. The work on *D. tabernella* also need to be done regarding the sex pheromone, as no synthetic chemical product is currently available to effectively attract the species. The first identification for *D. tabernella* based on cytochrome oxidase II was conducted in Colombia [30].

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