Recognition of Five Larval Instars in *Conotrachelus perseae* (Coleoptera: Curculionidae)[1]

Á. Castañeda-Vildózola[2], C.P. Illescas-Riquelme[3], J. Valdez-Carrasco[3], L.E. Cazado[4], J.R. Sánchez-Pale, and V. López-Martínez[5]

Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, Campus "El Cerrillo," Toluca, Estado de México, C.P. 50200, México

J. Entomol. Sci. 54(2): 1–8 (April 2019)

Abstract Head capsule widths of *Conotrachelus perseae* Barber (Coleoptera: Curculionidae) larvae extracted from avocado, *Persea americana* Mill. cv. Fuerte, were measured by computerized imagery. Head capsule widths ranged from 212 to 1,562 μm and, according to an analysis of frequency distribution using the HCAP program and Dyar's rule, formed five well-defined modal groups which suggests five larval instars. The mean head capsule widths were 264, 428, 642, 1,009, and 1,493 μm for the first through the fifth instars, respectively, with a probability of misclassification of instar below 3%.

Key Words avocado weevil, quarantine pest, larval growth, instar determination, Dyar's rule

The small avocado seed weevil, *Conotrachelus perseae* Barber (Coleoptera: Curculionidae), is reported from Guatemala, Honduras, Costa Rica, and Mexico (Barber 1919, Whitehead 1979). In Mexico, the distribution of *C. perseae* is restricted to an altitudinal range of 1,270 to 1,954 m above sea level (masl), in the biogeographic provinces of the central and southern parts of the country including the states of Mexico, Puebla, Morelos, Guanajuato, Hidalgo, Veracruz, Oaxaca, and Chiapas. These regions produce avocado commercially and also have native populations of other species of *Persea* and botanical varieties of avocado such as *P. americana* var. *drymifolia*, *P. americana* var. *guatemalensis*, and *P. americana* var. *americana* that can serve as host plants for this curculionid (Castañeda-Vildózola et al. 2015, Luna et al. 2017, Vázquez et al. 2015).

According to Peterson and Orden (2008), *C. perseae* is classified as a quarantine pest whose presence in commercial orchards presents a risk for the export of Mexican avocado to the United States. This initiated several studies aimed at an improved knowledge of the biology of this species (Llanderal and Ortega

---

[1]Received 06 November 2018; accepted for publication 08 November 2018.
[2]Corresponding author (email: acastanedav@uaemex.mx).
[3]Programa de Entomología y Acarología, Colegio de Postgraduados Campus Montecillo, Km 36.5 carretera México-Texcoco, Montecillo, Texcoco, Estado de México, México.
[4]Escuela de Agricultura y Sacarotecnia, Universidad Nacional de Tucumán, Horco Molle S/N, Yerba Buena, Tucumán, Argentina.
[5]Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos 62209, México.

1990, Payán-Arzapalo 2014, Salinas-Castro 1999). Castañeda-Vildózola et al. (2015) documented that *C. perseae* is nocturnal and that females perforate growing fruits to oviposit. The number of perforations ranges from one to six, with one to eight eggs deposited in each perforation. Larvae hatch from eggs, on average, in 7.0 d and feed in the seed, with as many as 24 larvae per seed. The entire larval stage lasts 25.0 d and, once concluded, last instars abandon the fruit and pupate in the soil. The prepupal and pupal stages last 24.4 and 16.33 d, respectively.

Integrated pest management programs require knowledge of the biology, ecology, and behavior of the target pest (Gold et al. 1999). Enumeration and characterization of the larval instars of the insect is an important component, but for spermatophagous species, such as *C. perseae*, the direct observation of larval molts is difficult, thus forcing reliance on indirect methods such as the measurement of head capsule width (Dyar 1890). Computer algorithms also have been developed for determination of larval instar based on head capsule widths (Logan et al. 1998, Merville et al. 2014).

Use of Dyar's (1890) rule is useful in determining larval instar of the curculionids *Lissorhoptrus kuscheli* O'Brien (Pantoja et al. 1999), *Anthonomus grandis* Boheman (Reardon et al. 2002), *Conotrachelus psidii* Marshall (Bailez et al. 2003), *Pissodes castaneus* (De Geer) (Panzavolta 2007), *Rhyssomatus subtilis* Fiedler (Cazado et al. 2014), *Curculio chinensis* Chevrolat (Zhi-Wen et al. 2015), and *Heilipus lauri* Boheman (Castañeda-Vildózola et al. 2016). Its application to weevils with intraspecific variation in the number of larval instars, such as *Cosmopolites sordidus* Germar (Gold et al. 1999), *Rhynchophorus ferrugineus* Olivier (Jaya et al. 2000), and *Scyphophorus acupunctatus* Gyllenhal (Valdés-Estrada et al. 2010), is much less reliable. Such variation is attributable to biological polymorphism, food availability and quality, and environmental factors like temperature, humidity, and photoperiod (Esperk et al. 2007).

Several studies of *C. perseae* in Mexico have reported number of larval instars observed, but the topic remains controversial. Payán-Arzapalo (2014) reported four larval instars, while Salinas-Castro (1999) and Cancino and Ponce (2000) observed five instars, and Llanderal and Ortega (1990) and Coria-Ávalos (1999) reported six. Our study was undertaken to clarify the number of larval instars occurring in a population of *C. perseae* collected from *Persea americana* Mill. cv. Fuerte avocado fruits cultivated in the state of Mexico.

Materials and Methods

Infested avocado fruits were collected from three orchards located in the community of Meyuca de Morelos, in the municipality of Coatepec Harinas, state of Mexico (N18.55°142′, W99.78°143′; 1,948 masl) at weekly intervals from January through October of 2015. In total, 1,756 infested avocados weighing a total of 311.23 kg were collected and transported to the laboratory to extract eggs and larvae. This is a zone of transition between temperate subhumid and warm subhumid climates. The mean annual temperature is 28°C (range, 11–37°C), with a mean annual precipitation of 1,321 mm.

In the laboratory, each oviposition hole was dissected using a camel hair brush to extract eggs; eggs were individually placed in petri dishes (9.0 × 1.0 cm) with

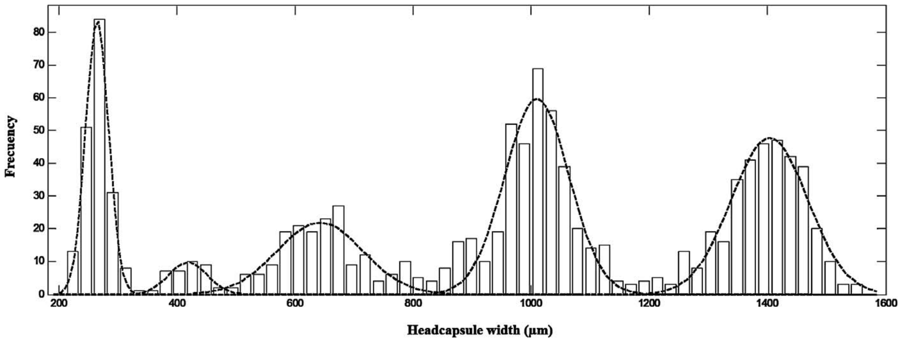

Fig. 1. Frequency distribution of the head capsule widths (µm) of *C. perseae* larvae as generated by the HCAP program.

absorbent paper moistened with distilled water as an incubation substrate. Eggs were monitored every 12 h, and newly emerged larvae were collected and preserved in 70% ethanol. Larvae also were extracted from seeds, placed in hot water (96°C), separated by size, and preserved in 70% ethanol.

Head capsule widths of the larvae were measured using computerized image analysis. Digital microphotographs were taken of the head capsules in frontal view with a PAXcam 3 Digital Microscope Camera (PAX-it, Villa Park, IL) mounted on a Tessovar III photomicroscope (Carl Zeiss, Obekochen, Germany). Images were saved in JPG format. The system was calibrated using an image of a ruler marked in gradations of hundredths of millimeters. The head capsules in the images were measured in micrometers from the outermost lateral edges (gena), using the program Image Tool 3.0 (Wilcox et al. 2002).

Head capsule width measurements were analyzed using the HCAP program developed by Logan et al. (1998), which is a generalization of the technique previously described by McClellan and Logan (1994). The precision of Dyar's rule was analyzed using linear regression by fitting the number of larval instars obtained with the natural logarithm of the mean head capsule width of each instar, following the method proposed by Gaines and Campbell (1935). A one-way analysis of variance using InfoStat (2008) software was conducted to test the statistical significance of the slope of the regression line.

Results and Discussion

Head capsules were measured on 1,148 larvae, including measurements of 56 neonates from eggs removed from fruits and incubated in petri dishes. Head capsule widths ranged from 212 to 1,562 µm which, according to the frequency distribution analysis, formed five well-defined modal groups and suggests five larval instars (Fig. 1). The descriptive statistics, including mean, standard deviation, and number of larvae observed in each stage, are included in Table 1.

The minimum and maximum values for each larval instar assigned by HCAP reduced the probability of overlap attributed to misclassification, which was below 3% (Table 1). The value of Dyar's constant was between 1.39 and 1.58, which

Table 1. Mean, range, and probability of misclassification for the measurements (width) of head capsules of the larval instars of *C. perseae* calculated using the HCAP program.

Instar	N	Mean ± SD (μm)	Range (μm)	Probability of Misclassification: i as $i-1$	i as $i+1$	Total	Dyar's Ratio
1	188	264 ± 19.50	189–330	0.000000	0.000759	0.000759	
2	36	418 ± 32.60	330–478	0.003627	0.032038	0.035666	1.58
3	182	642 ± 72.75	478–841	0.012343	0.003089	0.015432	1.54
4	389	1,009 ± 54.58	841–1,191	0.001028	0.000428	0.001456	1.57
5	353	1,403 ± 64.89	1,191–1,584	0.000544	0.000000	0.000544	1.39

suggests a geometric progression of growth of the head capsule of each larval instar as a signal of Dyar's (1890) rule. Gaines and Campbell (1935) concluded that a perfect geometric progression in head capsule growth could be represented by a simple linear regression, plotting the natural logarithm of head capsule width and the number of larval stages. In our study, the linear equation of the linear regression of head capsule width on the increase in each larval stage was highly significant and indicates that all head capsule measurements were satisfactorily classified as one of the five instars ($P = < 0.0001$, $R = 0.992$, $R^2 = 0.988$) (Fig. 2). The excellent fit of the model indicates no overlapping instars. The significant difference observed between head capsule width for each larval instar ($F = 13.188.28$; df = 3, 11; $P = < 0.0001$) confirms the results obtained from the linear regression.

The results of our study differ from those reported by Llanderal and Ortega (1990), Coria-Ávalos (1999), and Payán-Arzapalo (2014), but are consistent with those of Salinas-Castro (1999) and Cancino and Ponce (2000). Payán-Arzapalo (2014) measured only 357 larvae extracted from a mix of Hass, Fuerte, and criollo avocados (*P. americana* var. *drymifolia*); therefore, it is possible that the small sample size may have limited their conclusions of the occurrence of four larval instars. They also noted that their definition of instars 1 and 2 was uncertain. Llanderal and Ortega (1990) and Coria-Ávalos (1999) reported six larval instars in larvae collected from the Hass variety of avocado Coria-Ávalos (1999) also failed to include methods of measurement and analysis of head capsule measurements. While Llanderal and Ortega (1990) used similar methods of measurement and analysis as reported herein, their six modal groups had very narrow ranges among the instars, indicating the possibility of erroneous assignments of head capsule widths during their frequency distribution analysis.

Salinas-Castro (1999) and Cancino and Ponce (2000) suggested five larval instars in *C. perseae* collected from the Hass variety of avocado. Both studies used the same measurement methods but different techniques for analysis of head capsule widths. The results of those two studies suggest a slight overlap between the second and third instars. There was an observed 7% probability of

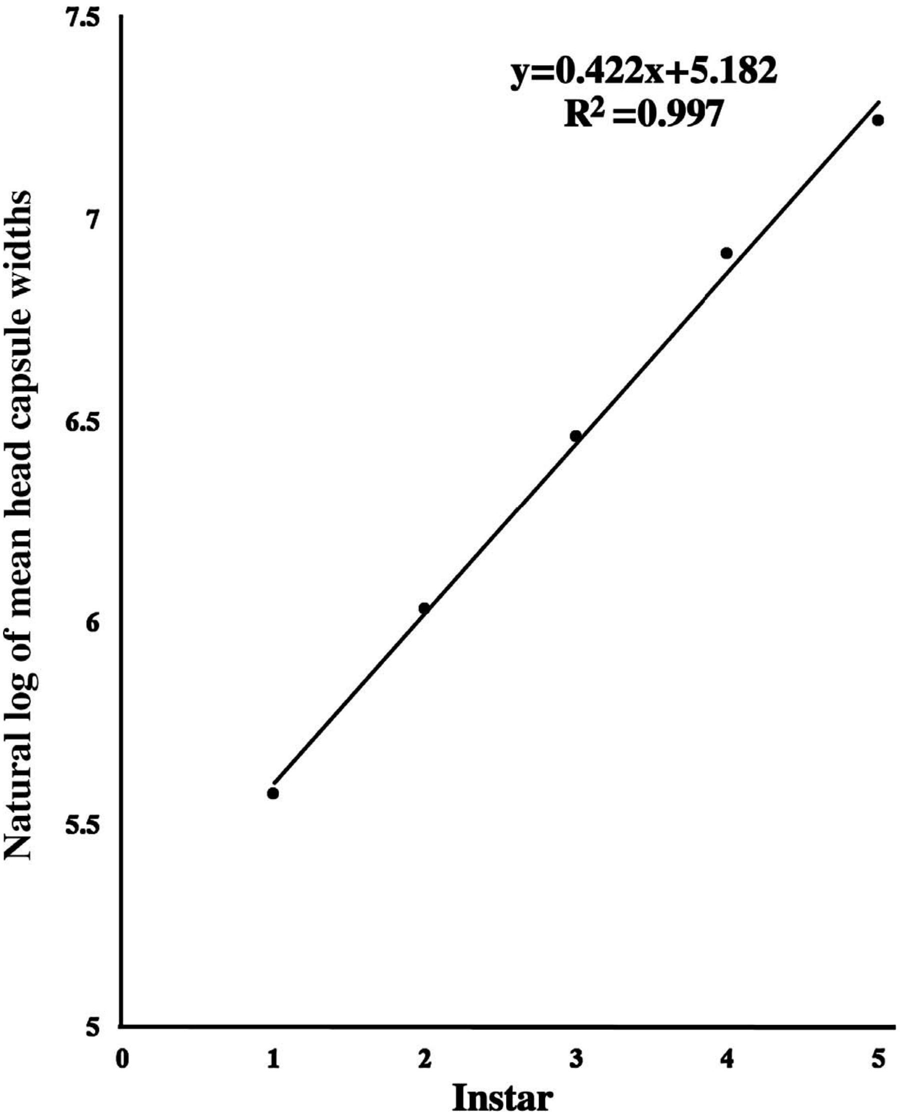

Fig. 2. Linear regression of the natural logarithm of the mean larval head capsule width on the instar stage of *C. perseae*.

misclassification in the Salinas-Castro (1999) study, while our study yielded a 3% probability of misclassification. The lower level of error in our study is attributed to a larger sample size of 1,148 larvae as compared with 841 in Salinas-Castro (1999).

Research in other species of *Conotrachelus* indicates interspecific variation in number of larval instars, but no intraspecific variation, as demonstrated by Bodenham et al. (1976), Raccette et al. (1992), Mendes et al. (1997), and Bailez et al. (2003), whose results showed four larval instars in *C. nenuphar* (Herbst), *C. humeropictus* Fiedler, *C. neomexicanus* Fall, and *C. psidii* Marshall, respectively. On the other hand, Muñiz and González (1982) and Tedders and Payne (1986) determined five larval instars for *C. dimidiatus* Champion and *C. schoofi* Papp.

Our study adds one more species to the list of *Conotrachelus* with five larval instars and is supported by the results of Salinas-Castro (1999) and Cancino and Ponce (2000). In addition, these results suggest that the genotype of the avocados used as food by the larvae (Hass cultivated in Michoacán; Fuerte cultivated in the state of México) does not lead to variation in the number of larval instars. As such, the discrepancies among different studies (i.e., Llanderal and Ortega 1990 and Payán-Arzapalo 2014) is more likely attributed to the small number of larvae measured and errors in the interpretation of the frequency distribution analysis of the head capsule width of *C. perseae* larvae.

Acknowledgments

We thank Fernando Becerril Garduño for help collecting fruits in the field; Dra. Lynna M. Kiere (Universidad Nacional Autónoma de México, México) for assistance in revision of our manuscript; Dr. Jorge E. Peña (University of Florida, USA) and Dr. Cristian Nava-Díaz (Colegio de Posgraduados, México) for critical reviews of this manuscript; Dr. J.A. Logan for providing the HCAP program; and Universidad Autónoma del Estado de México for financial support of this research through the project 3796/2014/CID.

References Cited

Bailez, E.O., V.M. Viana-Bailez, J.O.G. de Lima and D.O.D. Moreira. 2003. Life-history of the guava weevil, *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae), under laboratory conditions. Neotrop. Entomol. 32: 203–207.

Barber, H.S. 1919. Avocado seed weevils. Proc. Entomol. Soc. Wash. 21: 53–60.

Bodenham, J.R., E. Stevens and T.E. Thatcher. 1976. A cone weevil, *Conotrachelus neomexicanus*, on ponderosa pine in Colorado: Life history, habits, and ecological relationships (Coleoptera: Curculionidae). Can. Entomol. 108: 693–699.

Cancino, M.R. and S.R. Ponce. 2000. Ciclo de vida del "Barrenador pequeño del hueso del aguacate" (*Conotrachelus perseae* Barber) Coleoptera: Curculionidae), en condiciones de laboratorio, Pp. 674–679. *In* Stanford, S., A. Morales, J. Padilla and M. Ibarra (eds.), Memorias del XXXV Congreso Nacional de Entomología. Sociedad Mexicana de Entomología, Acapulco, Guerrero, México.

Castañeda-Vildózola, A., O. Franco-Mora, J.C. Reyes-Alemán, C. Ruiz-Montiel, J. Váldez-Carrasco and A. Equihua-Martínez. 2015. New distribution records of the small avocado seed weevil, *Conotrachelus perseae* Barber (Coleoptera: Curculionidae), in Mexico and notes on its biology. Coleop. Bull. 69: 267–271.

Castañeda-Vildózola, A., H. González-Hernández, A. Equihua-Martínez, J. Valdez-Carrasco, J.E. Peña, L.E. Cazado and O. Franco-Mora. 2016. Head capsule width is useful for determining larval instar in *Heilipus lauri* (Coleoptera: Curculionidae). Fla. Entomol. 99: 822–825.

Cazado, L.E., G.A. Van Nieuwenhove, C.W. O'Brien, G.A. Gastaminza and M.G. Murúa. 2014. Determination of number of instars of *Rhyssomatus subtilis* (Coleoptera: Curculionidae) based on head capsule widths. Fla. Entomol. 97: 639–643.

Coria-Ávalos, V.M. 1999. Ciclo de vida, fluctuación poblacional and control del barrenador de la semilla del aguacate (*Conotrachelus perseae* Barber, *Conotrachelus aguacatae* B.) (Coleoptera: Curculionidae) en Ziracuarétiro, Michoacán, México. Rev. Chapingo Ser. Hortic. 5: 313–318.

Dyar, H.G. 1890. The number of molts of lepidopterous larvae. Psyche 5: 420–422.

Esperk, T., T. Tammaru and S. Nylin. 2007. Intraspecific variability in number of larval instars in insects. J. Econ. Entomol. 100: 627–645.

Gaines, J.C. and F.L. Campbell. 1935. Dyar's rule as related to the number of instars of the corn ear worm, *Heliothis obsoleta* (Fab.), collected in the field. Ann. Entomol. Soc. Am. 28: 445–461.

Gold, C.S., P.S. Neyeme and R. Coe. 1999. Recognition and duration of the larval instars of banana weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) in Uganda. Afr. Entomol. 7: 49–62.

InfoStat. 2008. InfoStat, software estadístico. Manual del Usuario versión 2008. Grupo Infostat, FCA, Universidad Nacional de Córdoba. Editorial Brujas, Buenos Aires Argentina.

Jaya, S.T., R. Suresh, S. Sobhitha-Rani and S. Sreekumar. 2000. Evidence of seven larval instars in the red palm weevil, *Rhynchophorus ferrugineus* Oliv. reared on sugarcane. J. Entomol. Res. 24: 27–31.

Llanderal, P.L. and H.A. Ortega. 1990. Hábitos e identificación del barrenador pequeño de la semilla del aguacate (*Conotrachelus persae* Barber) en Ziracuaretiro, Michoacán. BS Thesis. Universidad Michoacana de San Nicolás de Hidalgo, Uruapan, Michoacán, México. 101 pp.

Logan, J.A., B.J. Bentz, J.C. Vandygriff and D.L. Turner. 1998. General program for determining instar distribution from head capsule widths: Example analysis of mountain pine beetle (Coleoptera: Scolytidae). Environ. Entomol. 27: 332–340.

Luna, A., V. López-Martínez, N.B. Pérez-De la O, D. Jiménez-García, R.W. Jones, A. Castañeda-Vildozola and C. Ruiz-Montiel. 2017. Actual and potential distribution of five regulated avocado pests across Mexico, using the maximum entropy algorithm. Fla. Entomol. 100: 92–100.

McClellan, Q.C. and J.A. Logan. 1994. Instar determination for the gypsy moth (Lepidoptera: Lymantriidae) based on the frequency distribution of head capsule widths. Environ. Entomol. 23: 248–253.

Mendes, C.B.A., B.P. Magalhães and O.S. Ohashi. 1997. Biología de *Conotrachelus humeropictus* Fiedler, 1940 (Coleoptera: Curculionidae), praga do cacaueiro e do cupuaçuzeiro na amazônia brasileira. Acta Amazon. 27: 135–144.

Merville, A., A. Vallier, S. Venner, A. Siberchicot, D. Fouchet, A. Heddi and M.C. Bel-Venner. 2014. Determining the instar of a weevil larva (Coleoptera: Curculionidae) using a parsimonious method. Eur. J. Entomol. 111: 567–573.

Muñiz, V.R. and R.E. González. 1982. *Conotrachelus dimidiatus* Champ., "el picudo de la guayaba" en Morelos, México. An. Esc. Nac. Cien. Biol. 26: 9–35.

Pantoja, A., L.M. Ramírez, L.E. Escalona, H. Bastidas and M.C. Duque. 1999. Number of instars of *Lissorhoptrus kuscheli* (Coleoptera: Curculionidae) from rice in Venezuela. Ann. Entomol. Soc. Am. 92: 236–238.

Panzavolta, T. 2007. Instar determination for *Pissodes castaneus* (Coleoptera: Curculionidae) using head capsule widths and lengths. Environ. Entomol. 36: 1054–1058.

Payán-Arzapalo, M.A. 2014. Bioecología del barrenador pequeño de la semilla del aguacatero *Conotrachelus persea* Barber (Coleoptera: Curculionidae) en Coatepec Harinas, Estado de México. MSc Thesis. Universidad Autónoma del Estado de México, Toluca, Estado de México, México. 61 pp.

Peterson, E.B. and D. Orden. 2008. Avocado pests and avocado trade. Am. J. Agric. Econ. 90: 321–335.

Raccette, G., G. Chouinard, C. Vincent and S.B. Hill. 1992. Ecology and management of plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), in apple orchards. Phytoprotection 73: 85–100.

Reardon, B.J., C.P. Suh and D.W. Spurgeon. 2002. Head capsule widths of larval instars of the cotton boll weevil. Southwest. Entomol. 27: 291–292.

Salinas-Castro, A. 1999. Biología del barrenador pequeño del hueso del aguacate *Conotrachelus perseae* Barber en Tacámbaro, Michoacán. MSc Thesis. Colegio de Postgraduados, Texcoco, Estado de México, México. 59 pp.

Tedders, W.L. and J.A. Payne. 1986. Biology, life history, and control of *Conotrachelus schoofi* (Coleoptera: Curculionidae) on pecans. J. Econ. Entomol. 79: 490–496.

Valdés-Estrada, M., C. Hernández-Reyes, M. Gutiérrez-Ochoa and L. Aldana-Llanos. 2010. Determination of the life cycle of *Scyphophorus acupunctatus* (Coleoptera: Curculionidae) under laboratory conditions. Fla. Entomol. 93: 398–402.

Vázquez, M.A., L. Cruz-López and E.R. Chamé-Vázquez. 2015. First record of *Conotrachelus perseae* (Coleoptera: Curculionidae) in Comitán, Chiapas, Mexico. Fla. Entomol. 98: 1252–1253.

Whitehead, D.R. 1979. Recognition characters and distribution records for species of *Conotrachelus* (Coleoptera: Curculionidae) that damage avocado fruits in Mexico and Central America. Proc. Entomol. Soc. Wash. 81: 105–107.

Wilcox D., B. Dove, D. McDavid and D. Greer. 2002. UTHSCSA Image Tool for Windows ver. 3.0. Univ. of Texas Health Science Center, San Antonio.

Zhi-Wen, L.I., H. Li-Hong, X. Jiao, M. Ling and Z. Ai-Ping. 2015. Determination of larval instars of the camellia weevil, *Curculio chinensis* (Coleoptera: Curculionidae). Acta Entomol. Sin. 58: 181–189.