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Article



Phylogeny of the genera *Euclidiodes* and *Hasodima* (Lepidoptera: Geometridae) and description of two new species from the Fray Jorge relict forest in northern Chile

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Abstract

The Fray Jorge National park contains the northernmost temperate relict forest of Chile (30°40'S), located over 1000 kilometers north of the rest of the coastal *Aextoxicon punctatum* (olivillo) communities of southern Chile. In this work we describe two new species of moths in the Fray Jorge relict forest belonging to the genera *Hasodima* Butler 1882 and *Euclidiodes* Warren 1895: *H. ediliacarmenae* Parra **sp. nov.** and *E. frayjorgeana* Parra **sp. nov.** The sister species of these new taxa are distributed in the central-southern zone of Chile, in plant associations where the olivillo is present. We hypothesize that the ancestor from which these species derived was widely distributed in association with coastal "olivillo" forests, which became restricted in distribution during interglacial periods, resulting in the isolation of these insects' populations, and their subsequent speciation.

Key words: Ennominae, Lithinini, Nacophorini, Hasodima ediliacarmenae, Euclidiodes frayjorgeana

Introduction

Species of geometrids from the genera *Euclidiodes* and *Hasodima* are distributed throughout the austral region of South America, in the temperate forest zone of central and southern Chile. This pattern of distribution coincides with the greatest diversity of geometrids on continental Chile south of 33° S (Parra 1997).

The genera *Euclidiodes* Warren 1895 and *Hasodima* Butler 1882 have five and three species, respectively (Parra 1999, Parra & Pascula-Toca 2003). In his review of the *Euclidiodes* Parra (1999) indicates that this genus is distinguished from other Lithinini by the "W" shaped gnathos and by the shape of the anellus process. According to Parra (1999) this genus is conformed by the species *agitata* (Butler 1882), *beechei* Parra 1999, *chiloensis* (Butler 1893), *meridionalis* (Wallengren 1860) and *ophiusina* (Butler 1882). Pitkin (2002) and Scoble & Hausmann (2007) transfer the following taxa to *Euclidiodes: chone* (Rindge 1986) (from *Yapoma*), *valdiviana* (Bartlett-Calvert 1893) (from *Incalvertia*), *xanthe* (Rindge 1986) (from *Duraglia*).

Parra & Pascual-Toca (2003) define *Hasodima* by the presence of a pseudouncus in the male genitalia. The unpaired furca and symmetrical juxta of the male genitalia are characters which allow this genus to be included in the Nacophorini tribe (Pitkin 2002). *Hasodima* includes the species *bartletti* Parra & Pascual-Toca 2003, *boreas* (Butler 1882) and *elegans* (Butler 1882).

Fray Jorge National Park contains the northernmost temperate forest of Chile (30°40'S), located over 1000 km north of the rest of the southern coastal olivillo communities in the country (Villagrán *et al.* 2004).

This forest became a relict as the semiarid matorral expanded, leaving the Fray Jorge forest restricted to the hilltops that received large inputs of water due to permanent fog during the interglacial periods of the Pleistocene. This fragmentation left Fray Jorge isolated from the other coastal forest communities of southern Chile (Villagrán *et al.* 2004) since at least between 2.5 million and 12 thousand years.

In this study we identify two new species of Geometridae from the genera *Hasodima* and *Euclidiodes* from samples collected in Fray Jorge National Park. Our aim is to clarify the phylogenetic relationships between species present in the relict forest of Fray Jorge and austral forests, as well as to discuss the importance of geographic isolation in the evolution of these two genera.

Materials and methods

The specimens examined in this study were obtained from samples made in Fray Jorge National Park within the framework of CASEB Program 4 "Biodiversity Conservation in Marine and Terrestrial Ecosystems of Chile", as well as from specimens deposited in the Zoology Museum of the Universidad de Concepción (MZUC). We examined a total of 213 adult specimens of the study species, analyzing the genital armature of both sexes of each species, and using standard methods for dissections and photographs. Nomenclature of the genitalia followed Klots (1979) and nomenclature of wing venation followed Scoble (1995).

Cladistic methods follow Nelson & Platnick (1981). We determined the generality of the characters of each transformation series by contrasting with an outgroup (Watrous & Wheeler 1981), specifically the sister groups *Oratha* for *Hasodima* and *Psilaspilates* for *Euclidiodes*. A listing of the transformation series and characters is presented in Tables 1 and 3, and the basic data matrices are presented in Tables 2 and 4. For more details on the species of *Hasodima* and *Euclidiodes* see Parra (1999) and Parra & Pascual-Toca (2003). The transformation series were treated as unordered series in *Hasodima* and ordered in *Euclidiodes*. Data were analyzed using the NONA 2.0 program (Goloboff 1993) implemented in WINCLADA 0.9.99.50.mexu (BETA) (Nixon 2002). The matrices were analyzed using 50 replicates, a hazard additional sequence, and a branch permutation of the type "tree bisection reconnection" (TBR) (Swofford & Olsen 1990). The characters were optimized using the fast optimization option. The support of the resulting groups was evaluated using bootstrap with 100 iterations, in order to estimate the probability of obtaining each of the phylogenetic groups resulting from the analysis.

Species descriptions

Hasodima ediliacarmenae Parra sp. nov.

Types. Holotype (male), Fray Jorge, 31 April to 1 May, 2005, Romina Villagrán- Mella coll. (UCCC-ZUC). Allotype (female), Fray Jorge, 31 April to 1 Mayo, 2005, Romina Villagrán- Mella coll., Museo de Zoología, Universidad de Concepción (UCCC-MZUC). Paratypes: 4 males, Fray Jorge, 31 April to 1 May, 2005, Romina Villagrán- Mella coll.; 1 male, Fray Jorge, 4 to 5 May, 2005, Romina Villagrán- Mella coll.; 1 female, Fray Jorge, 30 to 31 October, 2005, Romina Villagrán- Mella coll.; 1 male, 6 to 7 June, 2006, Romina Villagrán- Mella coll. (UCCC-MZUC)

Diagnosis. *Hasodima ediliacarmenae* and *H. bartletti* are very similar, being distinguished by the shape of the uncus, pseudouncus in male genitalia and the shape of the bursa copulatrix on the females. In *H. ediliacarmenae* the uncus and pseudouncus are digitiform, while in *H. bartletti* the subterminal zone of the uncus is enlarged and the pseudouncus is lanceolate. Female genitalia with anterior part of corpus bursae subpyriform in the new species whilst being spherical in *H. bartletti*.

Description. Adult (Fig. 1a). Head brown-green; brown-green, antennae bipectinate. Thorax brown-green; legs similarly colored, tibial spur formula 0-2-4; posterior tibias with hair tuft. Forewings brown-green;

antemedial and postmedial lines fine, dark brown-green and close to inner margin of the forewings, antemedial line proximally bordered by a whitish band reaching the basal edge of the wing; ground colour of terminal area lighter than the rest of the wing terminal dots brown; discal dot small, dark brown-green; postmedial line oblique; basal area with dark brown bar perpendicular to the body axis; underside of wings lighter brown-green, with discal dot and diffuse bands. Hindwings ochre, the terminal area dark brown; discal dot less apparent; terminal area dark brown. Abdomen conlorous to the wings, with dorsal tufts of scales between the third and seventh abdominal segment. Female. Similar to the male, with filiform antennae, posterior tibias without hair tuft.



FIGURE 1. Adult specimens of the genera *Hasodima* and *Euclidiodes*. (a) *H. ediliacarmenae* Parra **sp. nov.**, (b) *E. frayjorgeana* Parra **sp. nov.** Scale 1 cm.

Male genitalia (Fig. 2a–b). Uncus digitiform, apically rounded; pseudouncus digitiform; socii present, with setae; apical cleavage of valves subequal to the width of the valvula; costal margin of valva strongly sclerotized, subapically with digitiform projection; one cuspidiform projection in the center with numerous hairs; gnathos "V" shaped apically widened dentate and tapering tip; juxta subrectangular with two lateral processes in the form of a hook, 1/3 length of uncus; furca acicular, 4 times larger than lateral processes; region of the sacculus hollow; saccus subquadrangular; aedeagus straight, at ductus ejaculatorius constricted; vesica with a group of long and fine spines.

Female genitalia (Fig. 2c). Corpus bursae subpyriform, ductus bursae sclerotized with longitudinal furrows; signum large, peduncle and ensiform, five times smaller than the corpus bursae; peduncle the half of the ensiform body of signum; anterior apophyses 1/3 of the posterior apophyses; ductus seminalis arising from intersection between the corpus bursae and the ductus bursae; antrum quadrangular with sclerotized lateral areas, 16 times smaller than the corpus bursae.

Length of forewing. Measured from the base to the apex. Male: 14-15 mm (n = 6); female: 15 mm (n = 2).

Distribution. This species has only been collected in Fray Jorge National Park, in Coquimbo Region of Chile.

Flight period. Specimens of this species have been collected during the austral fall and spring, during the months of April, May, June and October, respectively.

Etymology. This species is dedicated to Edilia del Carmen, the wife of Luis E. Parra.

Euclidiodes frayjorgeana Parra sp. nov.

Types. 1 male, Holotype, Fray Jorge, 31 April to 1 May, 2005, Romina Villagrán- Mella coll. (UCCC-MZUC); 1 female, Allotype, Fray Jorge, 31 April to 1 May, 2005, Romina Villagrán- Mella coll., Museo de

Zoología, Universidad de Concepción (UCCC-MZUC). Paratypes: 8 males, Paratypes, Fray Jorge, 31 April to 1 May, 2005, Romina Villagrán- Mella coll.; 3 males, Paratypes, Fray Jorge, 1 to 2 May, 2005, Romina Villagrán- Mella coll.; 2 females, Paratypes, Fray Jorge, 31 April to 1 May, 2005, Romina Villagrán- Mella Coll.; 1 female, Paratype, Fray Jorge, 4 to 5 May, 2005, Romina Villagrán- Mella coll.; 1 male, Paratype, 6 to 7 June, 2006, Romina Villagrán- Mella coll. (UCCC-MZUC).

Diagnosis. *Euclidiodes frayjorgeana* and *E. ophiusina* are very similar, but are easily distinguished by the form of the anellus process, the cornuti of the aedeagus, the shape of the corpus bursae and the position of the signum. In male genitalia of *E. frayjorgeana* the anellus process is acicular and the aedeagus has a conical distal spine, while these traits are not present on *E. ophiusina*. In the female, the anterior part of the corpus bursae is ovoid in *E. frayjorgeana* and spherical in *E. ophiusina*; furthermore, the signum of *E. frayjorgeana* is positioned on a protuberance, while this trait is absent in *E. ophiusina*.



FIGURE 2. *H. ediliacarmenae* Parra **sp. nov.** (a) ventral view of male genitalia, (b) lateral view of aedeagus, (c) ventral view of female genitalia. Scale 1 mm.

Description. Adult (Fig. 1b). Head light brown, antennae simple, light brown. Thorax light brown, legs concolorous, tibial formula 0-2-4. Forewings, light ash brown, with traces of dark brown basal, medial and postmedial fasciae; basal and medial fasciae are barely perceptible towards costa, but form a conspicuous dark brown angle at the anal margin; the postmedial line is thin, dark brown, and positioned obliquely from the costal to the inner termen; subterminal fascia with an aristae inclined towards forewings termen on apical third. The region between the external margins and postmedial line has a diffuse, ash brown shadow which originates in the R_5 sector of the vein, diverging at the postmedial band and moving sinuously to the anal margin of the wing. Forewing underside ash brown, with darker diffuse areas corresponding to the transverse fascie. Hindwing upperside ash brown, underside light brown.

Male genitalia (Fig. 3a, b). Uncus long and narrow, hook-shaped, lateral socii at base of uncus short,

setose, digitiform, ½ length of valva; gnathos "W-shaped", tapering cetrally, with a series of small spines on each side; juxta "U-shaped", arms of the U are subtriangular; acicular anellus process, 1/3 length of valva; valva elongate, subrectangular, simple; cristae present, at tip near costa, approximately 4 to 5 setae on each side (removed by NaOH in figure 3a); saccus-vinculum subtriangular, anterior border deeply concave. Straight aedeagus, lanceolate at tip; vesica with a group of short and long spines, the latter being thin.

Female genitalia (Fig. 3c). Corpus bursae ovoid, ductus bursae tubular to conical, membranous in the posterior half and slightly sclerotized and striate on the anterior portion, ½ length of corpus bursae; circular, starred signum, located on the membranous protuberance on the lateroposterior part of the corpus bursae; the ductus seminalis arises ventrally from a small conical bag at the end of the corpus bursae; the antrum is sclerotized, subtriangular; posterior apophyses twice as long as the anterior.

Length of forewing. Measured from the base to the apex. Male: 13-14 mm (n=12); female: 15 mm (n=4). **Distribution.** This species has only been collected in Fray Jorge National Park, in Region IV of Chile.

Flight period. Specimens of this species have only been collected during the Austral fall, in the months of April and May.

Etymology. The name of this species refers to the type locality, Fray Jorge National Park, in Coquimbo Region of Chile.



FIGURE 3. *E. frayjorgeana* Parra **sp. nov.** (a) ventral view of male genitalia, (b) lateral view of aedeagus, (c) ventral view of female genitalia. Scale 1 mm.

Cladistic analysis of Hasodima

Oratha was used as outgroup because of its affinities with *Hasodima*, particularly the shape of the valva, gnathos and pseudouncus in the male genitalia. The most parsimonious tree obtained had a length of 29, a consistency index of 1, and a retention index of 1 (Figure 4). *Hasodima* is a monophyletic group, sustained by four synapomorphies (Figure 4, Tables 1, 2): 2(1) antemedial and postmedial fasciae reach the posterior margin of the anterior wings; 6(1) costal margin of the valves strongly sclerotized; 10(1) pseudouncus present; and 12(1) "V-shaped" gnathos. The species with the most ancestral traits is *H. boreas*, while *H. ediliacarmenae* presents more evolutionary novelties. *H. ediliacarmenae* and *H. bartletti* are the closest sister species, sharing 6 synapomorphies: 3(1) anterior wings are clear brown; 5(1) apical cleavage of the valves is sub-equal to their width; 13(2) subrectangular anellus; 14(1) anellus with lateral processes; 15(3) acicular furca of the anellus; and 19(2) size of the peduncle 1/2 of the signum. *H. bartletti* has three autapomorphies 4(2), 11(1) and 17(2), while *H. ediliacarmenae* has four: 4(1), 8(1), 17(3) and 18(2) (see Table 1).

TABLE 1. Polarized characters for phylogenetic analysis of Hasodima.

External traits

- 1. Male antennae: simple (0); bipectinate (1)
- 2. Antemedial and postmedial fasciae of forewings do not reach the posterior margin (0); do reach the posterior margin (1)
- 3. Color of the forewings: dark brown or dark brown-green (0); clear brown (1)
- 4. Location of the tufts of scales abdominal tergites: between segments 1 and 8 (0); between segments 3 and 7 (1); only on segment 3 (2)

Male genitalia

- 5. Apical cleavage of valves: ¹/₂ or less the width of valvula (0); subequal to width of valvula (1)
- 6. Costal margin of valva: slightly sclerotized (0); strongly sclerotized (1)
- 7. Apex of the costa of valva: pointed (0); blunt (1)
- 8. Region of the sacculus: whole (0); hollow (1)
- 9. Shape of uncus: hook (0); digitiform (1)
- 10. Pseudouncus: absent (0); present (1)
- 11. Shape of pseudouncus: spiniform (0); lanceolate (1); rhomboidal (2); digitiform (3)
- 12. Shape of gnathos: "U-shaped" (0); "V-shaped"(1)
- 13. Shape of the juxta: subquadrangular (0); subtriangular (1); subrectangular (2)
- 14. Lateral processes of the juxta: absent (0); present (1)
- 15. Shape of furca of juxta: cuneiform (0); espiciform (1); tubular (2); acicular (3)
- 16. Shape of posterior end of funda of aedeagus: blunt (0); pointed (1)

Female genitalia

- 17.- Shape of corpus bursae: coniform (0); elongate (1); spherical (2); subpyriform (3)
- 18.- Shape of signum: lanceolate (0); rhomboidal (1); ensiform (2)
- 19.- Length of peduncle relative to the signum: 1/16(0); 1/5(1); $\frac{1}{2}(2)$

Cladistic analysis of Euclidiodes

The related genus *Psilaspilates* was used as the outgroup (Parra & Hernandez 2009). We obtained 3 equally parsimonious trees with a length of 30, consistency index of 0.7, retention index of 0.55 and a rescaled consistency index of 38.5%. Figure 5 shows the strict consensus tree (length 32, consistency index 0.65, retention index 0.45). *Euclidiodes* is a monophyletic group sustained by one synapomorphy (see Figure 5,

Tables 3, 4): 6(1) presence of "W-shaped" gnathos. The species with the most ancestral traits is *E. chiloensis*, while *E. frayjorgeana* presents more evolutionary novelties. *E. frayjorgeana* and *E. ophiusina* are the closest sister species, sharing one synapomorphy: 10(2) subtriangular arms of the anellus. *E. ophiusina* is defined by the autapomorphy 11(1) acicular process of the anellus, while *E. frayjorgeana* presents three autapomorphies which easily distinguish it from its sister species: 11(2) caw shaped anellus process; 15(1) signum located on a protuberance of the corpus bursae; and 17(1) anterior portion of the corpus bursae is half the length of the posterior portion.

TABLE 2. Data matrix used in cladistic analysis of *Hasodima*. 0: plesiomorphic characters; 1, 2, 3: apomorphic characters; -: character not applicable.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Oratha significata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H. elegans	1	1	0	0	0	1	0	0	1	1	2	1	1	0	1	0	1	1	1
H. boreas	0	1	0	0	0	1	1	0	0	1	3	1	1	0	2	1	-	-	-
H. bartletti	1	1	1	2	1	1	0	0	1	1	1	1	2	1	3	0	2	1	2
H. ediliacarmenae	1	1	1	1	1	1	0	1	1	1	3	1	2	1	3	0	3	2	2

TABLE 3. Polarized characters for phylogenetic analysis of Euclidiodes.

External traits

- 1. Forewings fasciae: present (0); absent (1)
- 2. Forewings with dark triangular spots due to the convergence of medial and basal fasciae: absent (0); present (1)
- 3. Lighter coloration of (border of) forewing costa: absent (0); present (1)
- 4. Forewings with dark bars on the lateral veins of the cell: absent (0); present (1)
- 5. Subterminal fascia with an aristae inclined towards forewings termen on apical third: absent (0); present (1)

Male genitalia

- 6. Shape of the gnathos: "V-shaped" (0); "W-shaped" (1)
- 7. Position of spines on gnathos: widely distributed towards the lateral arms (0); concentrated in the middle region of the "W" (1)
- 8. Length of uncus with respect to valves: $\frac{1}{2}(0)$; $\frac{1}{3}(1)$
- 9. Shape of the juxta: subquadrangular (0); subrectangular (1); "U-shaped" (2)
- 10. Shape of arms of "U" of juxta: subrectangular (0); lobed (1); subtriangular (2)
- 11. Shape of anellus process: horn shaped (0); hooked (1); acicular (2)
- 12. Length of anellus process relative to length of valves: 1/3 (0); 1/5 (1)
- 13. Vesica of aedeagus: with group of spines (cornuti) (0); with a group of spines and a thick conical distal spine (1) Female genitalia
- 14. Shape of corpus bursae: elongate (0); spherical (1); ovoid (2)
- 15. Signum located on a protuberance: absent (0); present (1)
- 16. Shape of the signum: circular starred (0); subtriangular (1)
- 17. Length of ductus bursae relative to corpus bursae: subequals in length (0); $\frac{1}{2}$ of the length (1)

Discussion

Externally, *H. ediliacarmenae* and *E. frayjorgeana* are similar to their sister species *H. bartletti* and *E. ophiusina*, respectively. The observed external similarity, principally the wing pattern of the sister species, is

mainly due to the retention of ancestral homologous traits (parallelism), and does not necessarily demonstrate a relationship between these taxonomic entities. The phylogenetic analysis indicated homoplasies (parallelisms and reversion of traits) when these species attributes were analyzed. Nevertheless, examination of internal structures such as genitalia, provides diagnostic characters which allow the separation of these taxa in the context of a morphological pattern, indicating that they are related. Our introduction of these two new species is based on the apomorphies in the male and female genitalia: three of the four autopormorphies present on *H. ediliacarmenae* and three on *E. frayjorgeana*.

TABLE 4. Data matrix used in cladistics analysis of *Euclidiodes*. 0: plesiomorphic characters; 1, 2: apomophic characters; -: character not applicable.

Таха	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Psilaspilates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. agitata	0	1	0	0	1	1	1	1	2	0	0	1	1	0	0	0	0
E. beechei	0	0	1	0	0	1	1	1	2	1	0	1	0	2	0	1	0
E. chiloensis	1	0	0	0	0	1	0	1	0	-	0	0	0	-	-	-	-
E. frayjorgeana	0	1	0	0	1	1	0	0	2	2	2	0	0	2	1	0	1
E. meridionalis	1	0	0	1	0	1	0	0	1	-	0	1	0	0	0	0	0
E. ophiusina	0	1	0	0	1	1	0	0	2	2	1	1	1	1	0	0	0



FIGURE 4. Cladogram for species of *Hasodima*. Number above the circle corresponds to character observed, number below the circle represents the character state. Black circles: apomorphies, grey circles: character reversals. Number in parentheses indicates the degree of support (bootstrap).

The sister species of these two new taxa are distributed in the central-southern zone of Chile, in vegetational associations where *Aextoxicon punctatum* (olivillo) is found. Recent floristic and paleontological studies indicate that the relict forest of Fray Jorge is derived from a previously widely distributed vegetational formation which became increasingly fragmented and restricted towards the end of the Tertiary, with increased isolation as a result of climatic changes (Hinojosa & Villagrán 1997, Villagrán & Hinojosa 1997). Only the humid conditions in the glacial periods allowed continuous survival of this forest in the northern central zone of Chile (Villagrán *et al.* 2004). In this context, we hypothesize that the presence and restriction of the two newly described species to the relict forest of Fray Jorge is associated with *in situ* speciation. This presumes the existence of a widely distributed ancestor associated with this vegetation type during the last glacial period. From these parent species the two species described here would have been derived, as a result of the progressive fragmentation of their populations, due to the retraction of the olivillo forests during the postglacial period, and the simultaneous isolation of zones with high humidity, due to the presence of coastal fog, which duplicates the scarce precipitation in the form of rain -- makes the presence of the Fray Jorge forest

possible (e.g., del-Val et al. 2006), with a flora and fauna similar to that found in the temperate forests of southern Chile (e.g., Villagrán & Armesto 1980, Barbosa & Marquet 2002, Cornelius et al. 2000). In this context, we expect to find species restricted to other relict and isolated forests, such as Santa Inés, and hope to explore this in future studies.



FIGURE 5. Cladogram for species of *Euclidiodes*. Number above the circle corresponds to character observed, number below the circle represents the character state. Black circles: apomorphies; white circles: homoplasies; gray circles: character reversals. Number in parentheses indicates the degree of support (bootstrap).

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