

Reproductive system, mating behavior and basic ecology of an extremely rare tropical snail: *Drymaeus tripectus* (Stylommatophora: Bulimulidae)

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Abstract: Very little is known about the ecology and biology of *Drymaeus tripectus*, an extremely rare and endemic land snail species from Costa Rican highlands. I studied the ecology and reproductive biology of *D. tripectus* from April 2009 through June 2010 in an old forest, a young forest and a *Cupressus lusitanica* plantation in central Costa Rica. Every three months I visited each habitat and collected specimens in 20 random sampling plots (3x3 m² each). I observed the snail's activity and microhabitat preference in the field, and in the laboratory I recorded high definition videos of its mating behavior and analyzed reproductive morphology with light microscopy. The snail is more abundant in the old forest (0.017 ind./m²) and prefers leaves with little epiphyllous cover (0-25 % cover, chi-square test, $p < 0.0001$). During the dry season the snails become active between 20:00 pm and 8:00 am (chi-square = 22.65, $df=3$, $p < 0.0001$); they are inactive mainly during the afternoon (11:00 am to 16:59 pm). I found active individuals mostly on the upper side of leaves, where they feed (Chi-square = 6.76, $df=1$, $p = 0.0093$). Mating is unilateral, by shell mounting, with cryptic phallus intromission and without role switching or multiple mating. Its reproductive system is morphologically similar to that of *Drymaeus costaricensis*. Mating behavior is as expected for snails with high-spired shells, except for the lack of role switching. The density of *D. tripectus* is low even when compared with other endangered bulimulids. Rev. Biol. Trop. 64 (1): 55-68. Epub 2016 March 01.

Key words: land snail, reproductive anatomy, ecology, mating behavior, activity.

The tree snail *Drymaeus tripectus* Albers, 1857 is an extremely rare and nearly unknown tree snail of the family Bulimulidae. This species is closely related with *Drymaeus irazuensis* (Angas, 1878) and *Drymaeus gabbi* (Angas, 1878) (Pilsbry, 1899), as suggested by the adult red outer lip. These three species are endemic and restricted to the central Costa Rica highlands (Martens, 1802-1899; Pilsbry, 1899), an area impacted by urban expansion. Nothing has been reported about its ecology or biology (Martens, 1802-1899; Pilsbry, 1899; Barrientos, 2010). Breure and Eskens (1981) illustrated its radula and jaw, but its reproductive system was unknown until now.

In South America about 50% of the known snail species belong to this family (Breure & Borrero, 2008), and in Costa Rica (Central America) it is also one of the three most diverse families (Barrientos 2005; 2010). The genus *Drymaeus* has a total of 640 reported species that inhabit North, Central, South and Insular America, from sea level to 2 900 metres supra mare (msm) (Breure, 1979; Thompson, 2011). A great contribution to the understanding of *Drymaeus* taxonomy was made by Pilsbry (1897-1898, 1899), and more recently by Breure (Breure, 1974; 1979, Breure & Eskens, 1981; Breure & Borrero, 2008; Breure & Mogollón, 2010; Breure & Romero, 2012; among others).



Despite its high diversity and wide distribution, information on *Drymaeus* ecology and biology is scarce (Breure, 1979). Most observations are brief and included within systematic papers.

The Bulimulidae's reproductive anatomy is known for several species, including some Costa Rican *Drymaeus* species (Breure, 1974; 1979; Breure & Eskens, 1981; Beese, 2007; Beese, Armbruster, Beier, & Baur, 2009). It is reported as being simple externally and complicated internally, and as having a short flagellum and carrefour with multiple sperm storing tubules (Solem, 1955; Van Mol, 1971; Breure, 1974; Beese, 2007; Beese et al., 2009). Nevertheless, the mating behavior is not known and their mode of phallus intromission is unknown (Jordaens, Dillen, & Backeljau, 2009).

In this paper I have described the reproductive system, mating behavior and mode of phallus intromission of *D. tripectus*, and present basic information about its daily activity, abundance, demography and shell coloration.

MATERIALS AND METHODS

I selected three habitats with inclination over 40° in Reserva Forestal Río Macho (Cartago, Costa Rica):

Old Growth Forest with well-developed canopy and understory near the "El llano" water dam (9°45'56.07" N - 83°51'47.11" W - 1640 msm) (Digital Appendix 1) and Young Secondary Forest (Clark, 1996) left to natural succession for about 15 years, with a poorly developed canopy, and dense understory in some areas and grass in others (9°45'29.52" N - 83°51'23.27" W - 1684 msm) (Digital appendix 1). Both are located in a tropical lower montane wet forest area with ultisol humult soil.

Cupressus lusitanica Mill. 1768 plantation that has been without management for nearly 40 years and therefore has a poorly developed understory dominated by hardwood species (9°47'52" N - 83°51'51" W - 1309 msm) (Digital Appendix 1). Located in an area of tropical

humid premontane forest with inceptison dys-trandept soil.

I visited the old growth and young secondary forests in April, July and October 2009 and January 2010. The cypress plantation was sampled in July and October 2009 and January and April 2010.

On each sampling day I set a 200 m randomly located transect (chosen with a digital random number generator) at least 10 m away from any trail to avoid edge effect and away from tree gaps to avoid the effect of direct sun on litter (Camargo & Kapos, 1995). I also avoided sampling in the same transect twice. Each transect had 20 random 3x3 m² plots (Digital Appendix 2) where I collected all the snails I could find between 9:00 am and 2:00 pm.

In each plot and with the assistance of a student, I collected arboreal snails with a 50x50 cm² beat sheet (Digital Appendix 2) for about 7 min I gently beat branches, shrubs and seedlings up to 200 cm above ground and scrubbed tree trunks with a gardening glove. I also collected all leaf litter in a 50x50 cm² subplot (Digital Appendix 2).

I checked the leaf litter in the laboratory with magnifying glass and stereoscopic microscopes.

Empty shells can be misleading (Barrientos, 2000), so I only counted specimens collected alive, and I measured shell diameters with a Zeiss microstereoscopic scale at 16.25X. I classified specimens as newborn when only the embryonic whorls were present; juvenile when the embryonic and other whorls were present but periostome was not reddish or pinkish; and adult, when at least part of the periostoma was reddish or pinkish. On each habitat I calculated density by dividing total number of specimens by total area of the sampling plots.

I deposited specimens as vouchers to the University of Costa Rica Zoology Museum (MZUCR 220-01, MZUCR 220-02, MZUCR 220-03, MZUCR 223-01, MZUCR 225-01, MZUCR 227-01).

I observed daily cycle activity in specimens found in the primary forest in a 2 km long trail during seven sampling days in the

dry season (from December 2009 through April 2010). On each sampling day I walked along a trail for about an hour, and repeated the hike every three hours in a 24 hour period. In the first walk of the day, I numbered each specimen and used colour ribbons to mark trails, plants, stems and leaves so that I could follow each specimen individually (Digital Appendix 3). Additionally I marked specimen location on leaves with a felt-tipped marker to follow their displacement (Digital Appendix 3). In each case I took note of: hour, activity, behavior, part of the leaf (upper or lower side of the leaf), height on the plant above the ground, coverage of visible epiphyllous and weather ("rainy", "cloudy with wet soil", "cloudy with dry soil or sunny", I merge sunny and cloudy with dry soil categories as these two conditions appeared to be similar in terms of humidity of microhabitat of the snail in an old growth forest). I could not measure temperature and humidity. I classified time in four categories: 05:00 am-10:59 am (morning), 11:00 am-16:59 pm (afternoon), 17:00 pm-22:59 pm (night), 23:00 pm-04:59 am (dawn). I also classified time in day (05:00 am -16:59 pm) and night (17:00 am - 04:59 pm).

I classified inactive specimen behavior as: flattened, when tentacles were withdrawn and cephalopodium was extended and flattened out of the shell (Digital Appendix 3) or resting when cephalopodium was withdrawn in the shell. I considered specimens "active" when optical tentacles were extended and cephalopodium was extended but not flattened (Digital Appendix 3). I classified active specimen behavior as still or moving. I divided visible epiphyllous coverage, mainly moss and lichens, in four categories: 0-25 %, 25-50 %, 50-75 % and 75-100 %. I considered specimens "inactive" when optical tentacles were withdrawn in the body. Beside this, when possible I followed and measured the snail's trail, when not, I measured the shortest and simplest trail that the snail could have followed when they moved away from the original place.

Although I visited the primary forest study site several times during a year, I found adult

specimens only between March and April 2010. During this period I collected nine specimens on three different dates.

On three occasions I chose two individuals collected the same day and I kept them alone in the laboratory until the next day, when I put them together in a 946 ml container. Each pair of snails was filmed with a high definition Sony Handycam HDR XR 200 and took digital photographs to illustrate shell color variability in mating pairs. Intercourse was sometimes interrupted. In those cases, I considered the partner that split away first responsible of intercourse interruption. Some intercourse interruptions and separations after mating allowed me to observe the phallus of the male-acting partner. This allowed me to observe if phallus intromission was unilateral or simultaneous. After mating I isolated each specimen and kept them alive for one month.

I kept each specimen in a hermetic translucent plastic terrarium (10x10x11 cm³). Terraria had a 1 cm thick layer of wet soil from the Old Growth Forest. I provided food by putting medium age green leaves with epiphylls. I collected these leaves in the Old Growth Forest and kept them in a refrigerator at 5 °C to keep epiphytic algae alive for a longer time. I changed the leaves from the terraria every two or three days. I provided moisture and water by spraying the leaves before placing them in the terraria. I also removed excess water by decanting the terraria. Occasionally I put each couple together for at least one hour each time to observe if they mated again.

For anatomic analysis I dissected seven specimens: two with morphology A, three with morphology B and two with morphology C (Fig. 1). I found no variation, so considering how rare the species is I did not sacrifice additional specimens for this purpose. I extracted and dissected the receptaculum seminis with a stereoscopic microscope and observed the internal characteristic with a microscope. Vouchers for this part of the study are deposited in the University of Costa Rica Zoology Museum (MZUCR 218-01, MZUCR 218-02,

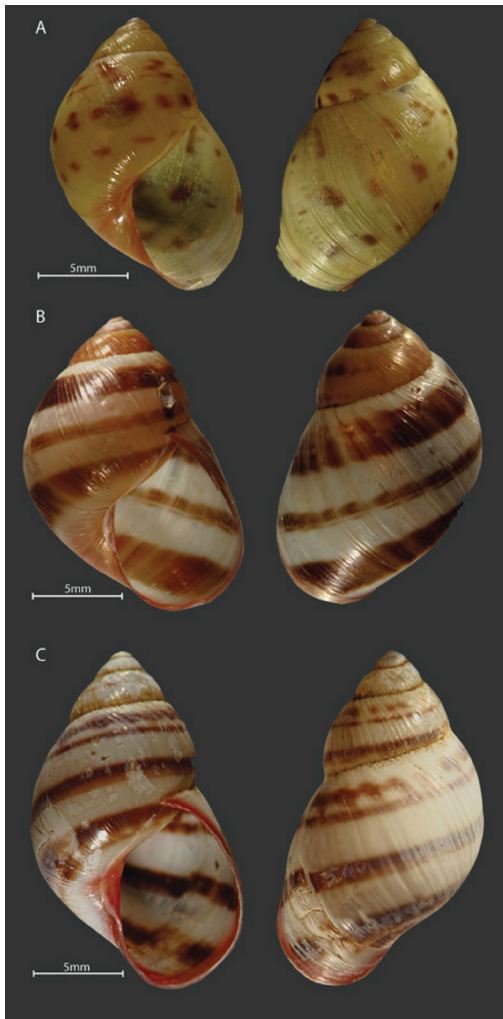


Fig. 1. Shell color pattern of *Drymaeus tripectus*. A and B have some white or translucent dots on body whorl similar to *Drymaeus irazuensis*. Mating was performed between the following color patterns: A with B., B. with C.

Fig. 1. Patrón de coloración de la concha de *Drymaeus tripectus*. A y B tienen algunos puntos translúcidos en la vuelta principal de la concha similares a los de *Drymaeus irazuensis*. Los apareamientos se presentaron entre individuos con los siguientes patrones de coloración: A con B, B con C.

MZUCR 218-03, MZUCR 219-02, MZUCR 219-03, MZUCR 224-01).

Overall, I found few living specimens because the species is so rare, therefore no inferential statistical analysis was possible. Specimens analyzed in the ecological part of

the study were not used for the anatomical or behavioural studies.

RESULTS

Shell color pattern variability: The shell color pattern of *D. tripectus* fits Pilsbry's description (1899), but some specimens have white or translucent dots characteristic of *D. irazuensis*. Specimens with and without translucent dots matted, as well as specimens with partial or complete red lip and with different color pattern (Fig. 1). I find the following color pattern proportions in the population (n= 11): Color pattern A= 45.5 %, B= 27.25 %, C= 27.25 %.

Reproductive system: Phallus and epiphallus large (average = 10.6 mm, maximum = 12.5 mm, minimum = 9.4 mm, sample size = 7), a little smaller than bursa copulatrix (average = 12.2 mm, maximum = 13.8 mm, minimum = 9.8 mm, sample size = 7). Phallus sub-cylindrical with a proximal thick, long sheath (average = 3.5 mm, maximum= 4.7 mm, minimum = 2.8 mm, sample size = 7) covering almost whole phallus (ca. 5/12 of phallus' length) (Fig. 2 A). Epiphallus relatively long slightly swollen and bent at the base above phallus sheath. Phallus a little wider (average = 0.8 mm, maximum = 1.1 mm, minimum = 0.6 mm, sample size = 7) than epiphallus (average = 0.7 mm, maximum = 0.8 mm, minimum = 0.6 mm, sample size = 7) due to phallus sheath. Flagellum tapering, "J" or "l" shaped, short (average = 1.7 mm, maximum = 2.7 mm, minimum = 1.3 mm, sample size = 7). Phallus retractor muscle short (average = 1.5 mm, maximum = 2.3 mm, minimum = 0.8 mm, sample size = 7) distally inserted in flagellum, originates on the pallial wall. Vas deferens: thin (average = 0.2 mm, maximum = 0.2 mm, minimum = 0.2 mm, sample size = 7), starting at epiphallus tip, inverted "u" shape at beginning, then runs very close or fasten to epiphallus and goes under phallus sheath; at about 1/3 phallus length bends "U-shaped" and crosses out phallus sheath (Fig. 2 B), once free, but covered by a very thin membrane or velum, bends again in inverted "U-shape"

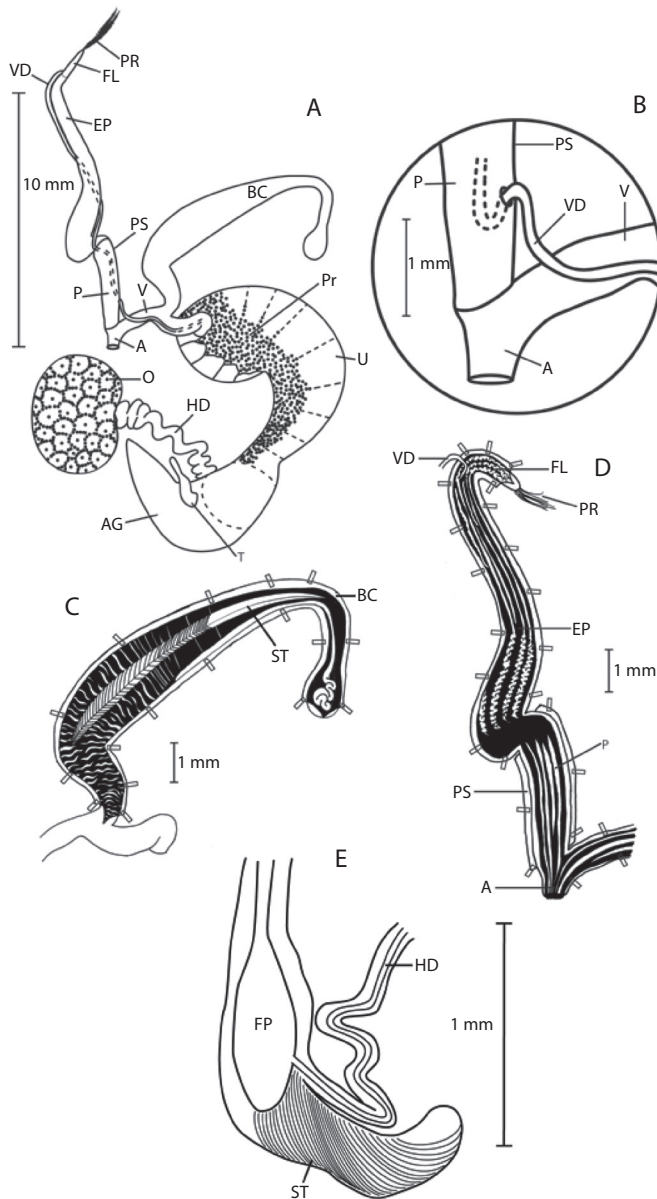


Fig. 2. The reproductive system of *Drymaeus tripictus*. A- Male and female reproductive organs. B- Detail of vas deferens getting through phallus sheath. C- Bursa copulatrix. D- Internal phallus pilasters. E- Receptaculum seminis. A= Genital atrium; AG= albumen gland; BC= Bursa copulatrix; EP= Epiphallus; FL= Flagellum; FP= Fertilization pouch; HD= Hermaphroditic duct; OD= Ovotestis duct; P= Phallus; PAG= Proximal Albumen Gland; Pr= Prostate; PS= Phallus sheath; PR= Phallus retractor muscle; SP= Sperm storing tubes; ST= Spermatophore; T= Talon or Receptaculum seminis; U= Uterus; V= Vagina; VD= Vas deferens.

Fig. 2. Sistema reproductivo de *Drymaeus tripictus*. A- Órganos reproductivos masculino y femenino. B- Detalle del vaso deferente atravesando la caperuz del falo. C- Bursa copulatrix. D- Pilastras internas del falo. E- Receptáculo seminal. A= Atrio genital; AG= Glándula de Albúmina; BC= Bursa copulatrix; EP= Epifalo; FL= Flagelo; FP= Bolsa de fertilización; HD= Ducto hermafrodita; OD= Ducto del ovotestis; P= Falo; PAG= Glándula Proximal de Albúmina; Pr= Próstata; PS= Caperuz del falo; PR= Músculo retractor del falo; SP= Tubos de almacenamiento del esperma; ST= Espermátóforo; T= Talón o Receptáculo seminal; U= Útero; V= Vagina; VD= Vaso deferente.

(Fig. 2 B); then it runs free along vagina; above spermathecal duct insertion, becomes fastened up to prostate base, where it is inserted. Bursa copulatrix rather broad (average= 1.5 mm, maximum=1.9 mm, minimum= 0.9 mm, sample size = 7) and subcylindrical at base, spool-shaped, connected to a globose end by a thin duct (average = 0.3 mm, maximum = 0.5 mm, minimum = 0.2 mm, sample size= 7) (Fig. 2 C). Bursa copulatrix is bent distally surrounding uterus and fastened to digestive gland. Its inner wall with abundant pilasters irregularly arranged in proximal part, transversally arranged and less dense in the middle. Globose end of bursa copulatrix contains a blind twisted duct. Spermatophores arch-shaped. Internal anatomy of phallus (Fig. 2 D). Flagellum with longitudinal irregular pilasters. Epiphallus distal end with longitudinal regular pilasters, vas deferens insertion goes in epiphallus lumen as a free duct (ca. 1/5 of epiphallus length). Pilasters become irregular near the epiphallus base. A relatively large space without pilasters separates epiphallus from phallus. Phallus with longitudinal regular pilasters. Atrium short (average = 0.7 mm, maximum = 0.8 mm, minimum = 0.3 mm, sample size = 6), inside with longitudinal regular pilasters (Fig. 2 D). Mid-sized vagina, longitudinal regular pilasters inside. Pilasters transverse after spermathecal insertion up to the uterus. Right optical tentacle runs between phallus and vagina. Prostate long (average = 8.3 mm, maximum = 9.4 mm, minimum = 7.0 mm, sample size = 7) adnate to uterus same length (Fig. 2 A). Hermaphroditic duct twisted, sometimes swollen, ovotestis very big (average = 5 mm, maximum = 6.3 mm, minimum = 3.9 mm), albumen gland from small to medium size. Receptaculum seminis or talon rather long (average = 1.8 mm, maximum = 2.3 mm, minimum = 1.3 mm, sample size = 7), digitiform, slender (average = 0.3 mm, maximum = 0.4 mm, minimum = 0.2 mm, sample size = 7) (Fig. 2 E). Receptaculum seminis with multiple sperm storing tubules that partially surround the fertilization pouch. Sperm-storing tubules spiral.

Mating: Mating only took place the first time that a pair was placed together (the specimens had been collected when alone on a particular tree) (sample size = 3 pairs). It began within one hour of being together, was performed by “shell mounting” and was unilateral with no role switching. Some snails defecated during mating. All specimens that mated had at least some reddish pigmentation on the aperture’s columella and parietal area, but coloration was sometimes absent on the outer lip. Mating can be divided in three stages: a) starting, stimulatory activity and gonopore search, b) intercourse and c) separation.

a) Beginning (Video 1), stimulatory activity and gonopore search (Video 2, Fig. 3 A): There was no obvious courtship; the male-acting partner simply slid onto the partner’s shell and achieved a mounting position (on the right side of the partner’s shell). The male-acting partner’s gonopore became slightly swollen and visible. The partner in the male role withdrew the right optical tentacle, turned the head and started stimulatory activity and search of the gonopore of the female-acting partner. The female-acting partner’s optical and labial tentacles were extended. This stage lasted around one minute. The female-acting partner may move on the ground with the partner in the male role riding on its shell. Stimulatory activity and gonopore search may be performed several times in the same mating event; they took place once that the male-acting partner was on the female-acting partner’s shell and every time that an interrupted intercourse restarted. The partner in the male role performed the stimulatory activity; with all the tentacles extended, started scrapping the shell and mantle collar of the female-acting partner with the radula during several 2 to 10 minute periods (with some breaks). This action looked as if the snail playing the male role were petting the partner’s head. Sometimes stimulatory activity was not performed and the partner in the male role simply began with the gonopore search. Gonopore search was performed by the partner in the male role. This behavior started

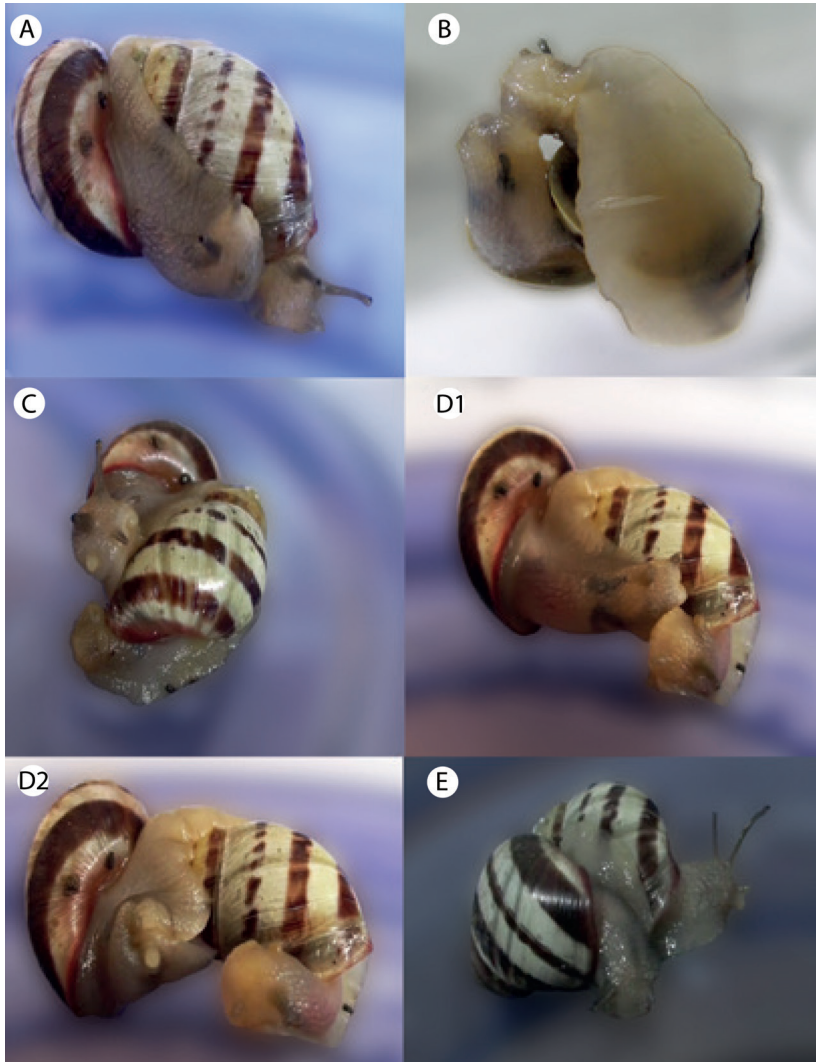


Fig. 3. Mating behavior. A- The male-acting partner is on the right side of the partner's shell. The head of the male-acting partner is upside down in an attempt to put gonopores in contact. B- Intercourse position. This position is kept only during 3 to 7 minutes if the intercourse is not complete and for more than an hour if it is complete. C- Phallus is partially everted, in this case the male-acting partner interrupted the intercourse. The head and tentacles of the male-acting partner are extended. D- Phallus is partially everted, in this case the female-acting partner interrupted the intercourse. D1- Female-acting partner scrapped the male's everted phallus with the radula. D2- The head and tentacles of the male acting partner are withdrawn. E- When the intercourse is over, the snails move apart without role switching.

Fig. 3. Comportamiento de apareamiento. A- El caracol que tiene el papel de macho está sobre el lado derecho de la concha de su pareja. El caracol que actúa como macho gira la cabeza tratando de poner los gonopores en contacto. B- Posición durante la cópula. Esta posición se mantiene sólo durante 3 a 7 minutos si la cópula no se completa y por más de una hora si la cópula se completa. C- El falo está parcialmente evertido, en este caso el caracol que actúa como macho interrumpió la cópula. La cabeza y tentáculos del caracol que actúa como macho están extendidos. D- El falo está parcialmente evertido, en este caso el caracol que actúa como hembra interrumpió la cópula. D1- El caracol que actúa como hembra raspó con la rádula el falo evertido del caracol que actúa como macho. D2- La cabeza y los tentáculos del caracol que actúa como macho están retraídos. E- Cuando la cópula termina, los caracoles se apartan sin realizar intercambio de papeles.

when the male-acting partner was on the right side of the partner's shell. The snail playing the male role withdrew optical and labial tentacles and turned its head to the left putting gonopores in a face to face position after some adjustment movements. I did not see an everted phallus during gonopore search. After that, the partner in the female role withdrew the tentacles and intercourse started.

b) Intercourse (Fig. 3 B): Once gonopores were put together, the position was maintained only for 3 to 7 minutes, if intercourse was interrupted; but for more than an hour if intercourse was completed. It seemed that intercourse was interrupted when phallus intromission was not completed in the first attempt; intercourse was interrupted several times during each mating event. During the three matings, I observed ten interruptions or unsuccessful penetrations attempts (interruptions by male-acting partner = 9; interruptions by female-acting partner = 1), but all three finished in what apparently looked as a complete successful penetration. No intercourse rejection was observed.

Intercourse interruption by partner in the male role (Video 3, Fig. 3 C): Intercourse was apparently interrupted more often by the partner in the male role; this can also be interpreted as unsuccessful penetration attempts. I observed up to five unsuccessful penetration attempts in a single mating event. It consisted of gonopore separation; in two occasions I saw the phallus partially everted, which was soon withdrawn. Whenever an intercourse was interrupted two activities could occur independently or in sequence: 1) resting, wherein the cephalopodium of the partner in the male role was kept straight laying on the partner's head for some minutes, and the optical tentacle of the male-acting partner may be withdrawn or everted; resting took from two to several minutes; 2) stimulatory activity and gonopore search, as already described. Meanwhile, the partner in the female role remained with tentacles withdrawn and motionless.

Intercourse interruption by partner in the female role (Video 4, Fig. 3 D): I only observed

one intercourse interrupted by the female-acting partner, who suddenly and quite abruptly turned its head backwards, separating gonopores, and scrapped its partner's partly everted phallus with the radula (Fig. 3 D1). The partner in the male role quickly withdrew its phallus and head (Fig. 3 D2), and stayed motionless for some seconds before restarting stimulatory activity and gonopore search. In this case a complete successful penetration was also achieved some minutes later.

Complete successful penetration: After several attempts (intercourse interruptions) the three matings got to an apparently complete successful penetration. Gonopores were put together and remained so for 60 to 75 minutes. The partner in the male role kept all tentacles withdrawn during the whole intercourse. I also observed some movements of the head of the male-acting partner, probably for penile adjustment, at the onset of mating. The female-acting partner kept optical and labial tentacles withdrawn most of the time.

c) Separation (Video 5, Fig. 3 E): Apparently, the male-acting partner starts separation; it withdrew its phallus and straighted up the head. These actions moved gonopores apart. The male-acting partner may: 1) keep tentacles withdrawn for up to 10 minutes resting on its partner's shell, and then extended its tentacles and slid down the partner's shell or 2) immediately extended the tentacles and slid down the partner's shell. The partner in the female role immediately everted tentacles and started moving away, even though the partner in the male role may be still on its shell. Once either of them gets active, it started feeding. Role switching was not performed.

All specimens were kept alive in the laboratory for about a month after their capture and mating activity, but none laid eggs.

Ecology: This endemic species is extremely rare: despite all the field work (120 hours) I only found 12 specimens in the primary forest, seven in the cypress plantation, and none in the secondary forest, therefore these results may be

considered preliminary, but particularly valuable considering that a species with such a small population is more likely to become extinct. Density was 0.017 ind/m² in primary forest and 0.0097 ind/m² in plantation. Hatching probably takes place in June and July, because newborns were found only on those months. There was a gradual size increase from July to April. In the forest I found three newborn snails in July (shell height 1.6 to 2.1 mm), four juveniles in October (4.0 to 4.6 mm) and three juveniles in January (5.3 to 10.2 mm), and two adults in April (14.8 to 15 mm). In the plantation: three newborns in July (1.8 to 2.1 mm) and four juveniles in October (2.3 to 8.0 mm).

Activity: As a result of the 92 activity records, I found that most snails were about 1.5 m above ground and on leaves with few visible epiphylls. During daytime they were mostly inactive, especially in the afternoon, and with the body flattened against the substrate. Weather and time did not affect their location on the leaf, but I found more active snails when the weather was cloudy and the soil wet. When they were active (mainly between two hours after sunset and two hours after sunrise) they moved on the upper side of leaves (a mean of 65 cm per day). Contrary to what I expected, inactive snails had no preference between the upper and underside of leaves (Digital Appendix 4).

DISCUSSION

More than a century ago Costa Rican naturalists had problems with European malacologists, who refused to believe the extreme rarity of most Central American land snails (Hilje, 2013, p. 563-564). But the truth is that even with teams of workers and long periods in the field, it is impossible to obtain large number of specimens, and therefore the biology of a vast majority of tropical and neotropical land snail species remains unknown (Barrientos, 2010). This ecological particularity of tropical malacofauna makes any data from the region especially valuable as it is very difficult to work and collect live specimens.

The reproductive system of *D. tripictus* is similar to that of *Drymaeus costarricensis* (L. Pfeiffer, 1862) from Matagalpa as described by Breure (Breure & Eskens, 1981), but phallus and phallus sheath are longer in this species. According to Pilsbry (1899) *D. irazuensis*, *D. gabbi* and *D. tripictus* form a closely related group; nevertheless, the anatomy of *D. irazuensis* and *D. gabbi* has not been described. Besides the red outer lip, the presence of white and translucent dots in *D. irazuensis* and *D. tripictus* is another possible indicator of their relationship.

Despite the high diversity of Bulimulidae, the reproductive behaviour of the family has not been reported before the present paper. However, the reproductive behavior of *Liguus*, a genus of a close family (Orthalicidae), is similar to *D. tripictus* as it is also unilateral by shell mounting (Davison & Mordan, 2007). Mating of *D. tripictus* is unilateral, by shell mounting, without role switching and with cryptic phallus intromission. This fits with what is generally expected for high-spired shell species, except for the fact that I did not observe the role switch (Jordaens et al., 2009).

When a hermaphroditic species has unilateral mating, partners often switch roles after a round of mating (Davison & Mordan, 2007), but this was not the case for *D. tripictus*. Previous studies report *Drymaeus papyraceus* (Mawe, 1823) as having a carrefour with multiple sperm storing tubules (van Mol, 1971; Beese, 2007; Beese, Armbruster, Beier, & Baur, 2009) similar to my findings for *D. tripictus*. The presence of a complex carrefour is related to multiple mating and cryptic choice by the partner in the female role (Gómez, 2001; Beese, 2007; Beese et al., 2009). Therefore, contrary to my observations, *D. tripictus* anatomy predicts the occurrence of multiply matings. The search costs model (Heller, 2001) and the abundance of *D. tripictus* could explain why no courtship was observed and the willingness to mate with the first partner met, but it does not explain why specimens did not have reciprocal mating or mated more when possible.

This species, like other members of the genus, has a well-developed phallus. It is everted once gonopores are closely pressed to each other, giving a cryptic phallus intromission. This is performed by a simple turn of the male's cephalopodium, allowing direct gonopore contact. Neck twisting is similar to *Oxyloma retusum* (Succineidae) (Örstan, 2010). Neck twisting has been also reported for *Euglandina rosea*, but in this case the snail in the lower position is the one that twists the neck to finish in a head to head position (Cook, 1985).

Either partner may interrupt the intercourse, but intercourse is more commonly interrupted by the partner in the male role, which at first glance has a more active role. The behavior described in this paper points out that it is also possible that the partner playing the female role can also interrupt intercourse. Webb (1948) suggested that biting during mating in *Zonitoides* (*Ventridens*) is a defense reaction against dart shooting. In *D. tripictus* there is no dart shooting and biting during mating seemed to be a "violent" reaction to something that was unexpected or out of the ordinary during intercourse. It cannot be interpreted as mate rejection because some minutes later intercourse with the same partner was accepted and completed. It also cannot be interpreted as stimulatory activity because the reaction of the partner playing the male role was the same as when a sharp object hurts the snail. Theory has been developed considering the male-acting partner as the "harming" partner in simultaneous hermaphrodite species (Schilthuisen, 2005; Koene, 2006; Preece, Mao, Garrahan, & Davison, 2009), but further analysis should also consider mating behavior, sperm storage and a more active role of the female-acting partner.

Intercourse interruption by the partner in the male role may be the results of an incomplete phallus intromission and of chemical or other internal signals that make the partner interrupt intercourse. The female-acting partner may play an important role in intercourse interruption by the male-acting partner: the right tentacle of the female-acting partner may not be in the correct position or the phallus intromission

may be internally avoided. More analyses are needed to understand if the stimulatory activity (scrapping shell of the female-acting partner with the radula) improves male- or female-acting partner performance.

Although understanding population density is crucial for conservation, most neotropical literature does not analyze species abundance (Barrientos, 2010). Findings of this study show that *D. tripictus* density is low in comparison with other neotropical wild species, about: 200 times less abundant than *Polymita muscarum* (Bidart, Fernández-Milera, Fernández, & Osorio, 1995) in Cuba; 10 times less abundant than *Liguus fasciatus* and *Zachrysis guanensis* (Poey, 1857) in Cuba (Alfonso, Berovides, & Rodríguez, 1991; Fernández-Velázquez & Berovides-Alvarez, 2001). In New Caledonia the genus *Placostylus* Beck 1837 (Bulimulidae) is considered endangered; nevertheless their density is higher than the density of *D. tripictus* (0.017 ind/m²): *Placostylus fibratus* Martyn, 1789 between 0.025 and 0.18 ind/m², *Placostylus porphyrostomus* (Pfeffer, 1851) between 0.009 and 0.115 ind/m², *Placostylus hongii* (Lesson, 1830) between 0.78 and 8.1 ind/m², *Placostylus bollonsi* Sutter, 1908 between 0.15 and 0.35 m² and *Placostylus bivaricosus* (Gascoin, 1854) 0.24 ind/m² (Brescia, Pöllabauer, Potter, & Robertson, 2008).

For conservation purposes we need to understand which biotic and abiotic habitat characteristics allow the presence of a particular species, especially for those that are endemic. Canopy may favour *D. tripictus*, because it occurs in the primary forest and the cypress plantation, but not in secondary forest (where the canopy is thinner). Land molluscs are greatly affected by humidity and vegetation, therefore, litter, moisture, canopy coverage and treefall gaps should be considered in future analyses (Alvarez & Willig, 1993; Pérez, 1994; Cowie, Nishida, Basset, & Gon, 1995; Horsák & Hájek, 2003; Barrientos, 2012).

Information on longevity and life cycle of *Drymaeus* species have not been reported (Beese, 2007). According to findings in this paper, it is possible that *D. tripictus* life cycle

is synchronized with seasons. Despite the few data, a possible life cycle pattern, that must be confirmed with more data, is that hatching occurs in the rainy season (June-July), mating takes place 10 months later during the dry season's second half (March through May) and eggs are probably laid in late May and June at the rainy season beginning. Season synchronization is reported for other American orthaliids and helminthoglyptids (Bidart, Fernández & Iglesias, 1988; Bidart, Osorio, & Reinaldo, 1992; Cuezco, 1993; Fernández-Velázquez & Berovides-Alvarez, 2001). Data do not allow me to establish the species longevity, but they probably live at least one year.

Species with long life span are able to store sperm for long periods (Gómez, 2001), therefore mating can take place months before they lay eggs. This may explain why no egg laying was observed in the specimens kept in the laboratory.

I found more specimens when the weather was cloudy and the soil wet. They seem to avoid activity during showers, when the impact of raindrops can force them to withdraw the tentacles or even knock them off leaves. My observations do not support Breure's assumption about bulimulinae's activity during heavy tropical showers (Breure, 1979).

Time of day was important to their activity and displacement, like reported for *P. fibrates*, another bulimulid (Brescia et al., 2008). This survey was done in the dry season, when the driest part of the day was the late afternoon because soil and litter moisture had been evaporating for several hours. At this dry period the snails became inactive and flattened against the substrate. Probably, relative moisture was not low enough to induce the behavior called *Resting* in this study.

In relation with their displacement, snails preferred to move during the night (two hours after sunset) and until early morning (two hours after sunrise). This is the time of the day when temperatures drop, and consequently relative moisture rises. There are few studies on displacement and movement in native tropical snail species. Many studies deal with snail

speed (Hyman, 1967), and daily displacement is known for a few species, mainly those of agricultural importance. Some species have been reported to move less than 5 m in their whole life (Pfenninger, Eppenstein, & Magnin, 2003), while slugs of agricultural importance have been reported to move 1 to 4 m a day (Stephenson, 1968). To better understand snail movements, future analyses should consider shell size, time of the day, season and relative humidity.

My results showed that *D. tripectus* prefer leaves with a small coverage of moss and lichens. Those leaves are probably covered with algae and cyanobacteria (which the snails forage on) that cannot be seen with the naked eye. This also explains why snails are more active while on the upper leaf; algae photosynthesize and therefore they must be more abundant on the upper side of leaves.

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RESUMEN

Sistema reproductivo, comportamiento de apareamiento y ecología básica de un caracol tropical extremadamente raro: *Drymaeus tripectus* (Stylommatophora: Bulimulidae). Se conoce muy poco sobre la ecología y biología del caracol terrestre *Drymaeus tripectus*. Se trata de una especie extremadamente escasa y endémica de las zonas altas de Costa Rica. Estudié la ecología y biología reproductiva de *D. tripectus* de abril 2009 a junio 2010 en un bosque maduro, un bosque joven y una plantación de *Cupressus lusitanica* en Costa Rica. Cada tres meses visité esos hábitats y recolecté especímenes en 20 puntos de muestreo (cada uno de 3x3 m²). Estudié actividad y preferencias de hábitat en la naturaleza. En el laboratorio

grabé en alta definición el comportamiento de apareamiento y analicé la morfología reproductiva con un microscopio estereoscópico de luz. El caracol es mas abundante en el bosque maduro (0.017 ind/m²) y prefiere hojas con poca cobertura de epífilos (25% o menos de cobertura, chi-cuadrado, $p < 0.0001$). En la época seca, se activan entre las 8:00 pm y las 8:00 am principalmente (chi-cuadrado = 22.65, $p < 0.0001$). En la tarde se inactivan (11:00 am to 16:59 pm). Los individuos activos se encuentran principalmente en el haz de las hojas (Chi-square = 6.76, $df = 1$, $p = 0.0093$), que es donde se alimentan. El apareamiento es unilateral por “montaje de concha”, no hacen cambio de roles sexuales y la intromisión fállica es críptica. Su sistema reproductivo es morfológicamente similar al de *Drymaeus costarricensis*. El comportamiento de apareamiento es como se espera de un caracol de concha con espira alta, excepto por la ausencia de cambio de roles. La densidad de *D. tripectus* es mas baja que la de otros bulimulidos considerados en peligro.

Palabras clave: molusco terrestre, anatomía reproductiva, ecología, comportamiento de apareamiento, actividad.

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DIGITAL APPENDIX

Video 1. *Drymaeus tripictus* male-acting partner slides on the shell of the snail acting in the female role in a “mounting shell” position. Sometimes, the female-acting snail goes on sliding a couple of minutes as shown in this video.

Video 1. El caracol *Drymaeus tripictus* que actúa como macho se desliza sobre la concha del caracol que actúa como hembra hasta alcanzar la posición conocida como “montaje de conchas”. En ocasiones, el caracol que actúa como hembra continúa deslizándose sobre el sustrato por algunos minutos como se muestra en este video.

Video 2. *Drymaeus tripictus* gonopore search. Male-acting partner withdraws tentacles and turns the head. Then stimulatory activity is performed by scrapping the shell of the female-acting partner with the radula. Female-acting partner stops sliding and the snail acting in the male role reaches the desired position in which both gonopores are side-by-side; no everted phallus can be seen from the outside. Snail in the female role withdraws the right optical tentacle; other tentacles may be withdrawn, full or partially extended.

Video 2. Búsqueda del gonoporo en *Drymaeus tripictus*. El caracol que actúa como macho retrae los tentáculos y gira la cabeza. Luego estimula al caracol que actúa como hembra raspándole la concha con la rádula. El caracol que actúa como hembra deja de deslizarse por el sustrato y el caracol que actúa como macho alcanza la posición deseada en la cual ambos gonoporos quedan en contacto; externamente no se puede ver el falo evertido. El caracol que actúa como hembra retrae el tentáculo óptico derecho; los demás tentáculos pueden estar parcial o completamente extendidos.

Video 3. *Drymaeus tripictus* intercourse aborted by the snail in the male role, followed by stimulatory activity. When intercourse is aborted, the male may scrape the shell of the snail in the female role before trying a new gonopore search and intercourse attempt. When the intercourse is aborted, the everted phallus can sometimes be seen for a second before it is withdrawn.

Video 3. La cópula de *Drymaeus tripictus* fue abortada por el caracol con el papel masculino. Esta interrupción es seguida por actividades de estimulación. Una vez que la cópula es abortada, el caracol que actúa como macho puede raspar con su rádula la concha de su pareja antes de realizar una nueva búsqueda del gonoporo y de intentar una nueva cópula. Ocasionalmente, cuando se interrumpe la cópula se puede observar el falo parcialmente evertido del caracol que actúa como macho durante algunos segundos antes de que sea retraído completamente.

Video 4. *Drymaeus tripictus* intercourse aborted by the female-acting partner. After the snail in the female role scrapes the male-acting partner’s phallus, the snail in the male role withdraws cephalopodium for some seconds, then it remains in resting position for some minutes. Resting can be performed after an intercourse is aborted or after a successful intercourse.

Video 4. Cópula de *Drymaeus tripictus* abortada por el caracol que actúa como hembra. Después de que el caracol en el papel femenino raspa con la rádula el falo del caracol que actúa como macho, el caracol en el papel masculino retrae el cefalopodio durante algunos segundos, después permanece en posición de descanso por algunos minutos. Los caracoles suelen descansar después del apareamiento tanto cuando la cópula es interrumpida como cuando es exitosa.

Video 5. *Drymaeus tripictus* intercourse separation. After mating snails start eating.

Video 5. Finalización de la cópula en *Drymaeus tripictus*. Después del apareamiento los caracoles se alimentan.

See Digital Appendix / Ver Apéndice digital