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REVISION OF THE REPRODUCTIVE MORPHOLOGY OF THREE *LEPTAXIS* SPECIES (GASTROPODA, PULMONATA, HYGROMIIDAE) AND ITS IMPLICATION ON DART EVOLUTION

Joris M. Koene[1] & Igor V. Muratov[2]

ABSTRACT

Many species of land snails have one or more sharp, calcareous "love darts" that are used to stab the partner during mating. These darts are produced and stored in specialized organs called stylophores. Because their number and position varies among species, stylophores are often used for identification and classification, especially in the family Hygromiidae. Having several stylophores, and thus several darts, is presumably the ancestral state from which species with one stylophore evolved. Species with small accessory sacs or rudimentary stylophores located above the functional stylophore are therefore thought to represent intermediate forms between species with double and single stylophores. We investigated the stylophores, darts, and associated reproductive organs of three species of the hygromiid genus *Leptaxis* – *L. erubescens*, *L. nivosa* and *L. undata*. In all the specimens of the investigated species, a small sac located just above the stylophore was found to be present. We conclude that this previously overlooked organ represents a rudiment of a stylophore, leading us to conclude that *Leptaxis* should be considered as an intermediate form in the evolution towards a single stylophore in the Hygromiidae.

Keywords: love dart, dart sac, stylophore, snail, stylommatophora, Helicoidea, rudiment.

INTRODUCTION

In the reproductive system of many hermaphroditic land snail species, one or more sharp, calcareous structures are present. When these are produced in a specialized organ, the stylophore (also referred to as dart sac), they are called darts. In many species, these "love darts" are stabbed through the partner's skin during mating (Adamo & Chase, 1990; Reyes Tur et al., 2000). In *Helix aspersa* (Müller, 1774) – often called *Cornu aspersum*, *Cryptomphalus aspersus*, or *Cantareus aspersus* – this "dart shooting" results in the transfer of an allohormone that inhibits sperm digestion and thereby increases sperm storage and fertilization success (Koene & Ter Maat 2001; Koene & Chase, 1998a; Rogers & Chase, 2001, 2002; Landolfa et al., 2001; Landolfa, 2002).

Recently, a comparative study demonstrated that the evolution of darts may be driven by sexual conflict (Koene & Schulenburg, submitted), thus explaining the diversity in number and shape of darts. For example, *Trichia* has two conical darts without blades (Schileyko, 1978a); *Leptaxis* and *Hygromia* each have one dart with two (differently arranged) blades (respectively: Spence, 1911; Giusti & Manganelli, 1987); *Helix* has a dart with four blades (Hasse et al., 2002); and *Monachoides* has one dart with seven blades (Koene & Schulenburg, submitted). Some species, such as *Cepaea nemoralis* and *C. hortensis*, which are otherwise remarkably similar, can most easily be distinguished by the shape of their darts (Kerney et al., 1983). Despite the large diversity in shapes, darts are rarely used for taxonomic purposes. Conversely, stylophores are traditionally used for identification and classification of land snails within the superfamily Helicoidea (Nordsieck, 1987; Schileyko, 1989). Species with one stylophore are thought to have evolved from ancestral species bearing several stylophores (Schileyko, 1989). When more than one stylophore is present, different arrangements are possible. Several stylophores can be arranged around the vaginal duct (e.g., *Humboldtiana*: Thompson & Brewer, 2000). Two pairs of stylophores can be present on opposing sites of the vaginal

[1]Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands; joris.koene@falw.vu.nl
[2]Department of Malacology, The Academy of Natural Sciences, Philadelphia, Pennsylvania, USA

duct (e.g., *Trichia*: Schileyko, 1978a). Alternatively, only one pair of stylophores can be present (*Hygromia*: Giusti & Manganelli, 1987). In these latter two cases, within each stylophore pair, the stylophore that is furthest away from the genital opening normally does not contain a dart, but see Taniushkin et al. (1999) for a possible case of atavistic development of darts in the upper stylophores of *Xeropicta krynickii* (Krynicki, 1833).

There are also species with morphologies that clearly represent intermediate stages between the above-mentioned forms. In such cases, a stylophore has become reduced in size and no longer produces a dart; such a rudimentary organ is then often referred to as an accessory sac (Nordsieck, 1993), internal dart sac (Giusti & Manganelli, 1987), or upper stylophore (Schileyko, 1989). Because these terms all describe the same organ, probably at different stages of reduction, we have chosen to use the term upper stylophore throughout the rest of this paper. Obviously, these intermediates provide important information about the course of evolution of the stylophore(s). Because rudimentary organs can be greatly reduced in size, they have sometimes been overlooked in previous studies. This is the case for the genus *Leptaxis*, which is why we redescribe the morphology of the stylophores, darts and associated reproductive organs of three species of this genus.

MATERIAL AND METHODS

Species of the genus *Leptaxis* inhabit Macaronesia, which includes 32 islands grouped in five major archipelagos: Azores, Canaries, Cape Verde, Madeira, and Selvagens

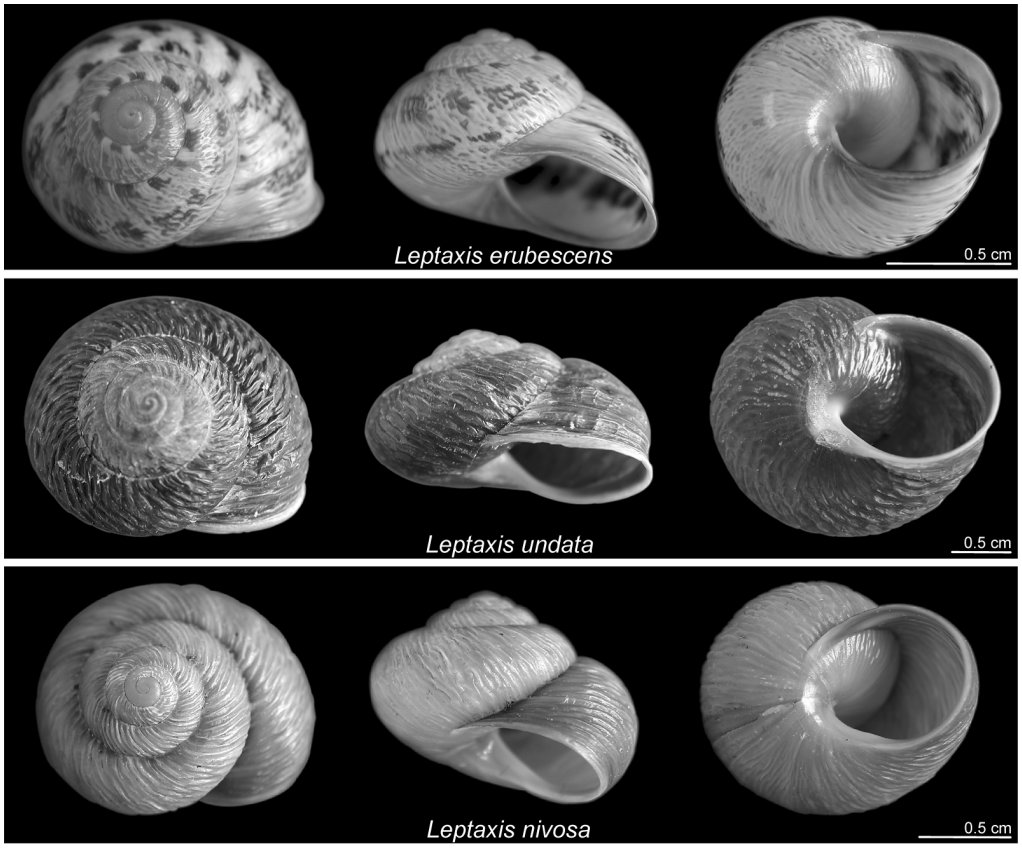

FIG. 1. Shells of the investigated *Leptaxis* species.

(Mitchell-Thomé, 1976). We have focused on the reproductive morphology of species endemic to the Madeiran archipelago. Among these, *Leptaxis erubescens* (Lowe, 1831) is the only species of this genus that occurs on all the different island groups – Madeira, Porto Santo, and The Desertas – of this volcanic archipelago (Cook, 1996). The other *Leptaxis* species are confined to one of the island groups (Cook, 1996). Of these species we investigated, *L. undata* (Lowe, 1831) from Madeira and *L. nivosa* (Sowerby, 1824) from Porto Santo. Figure 1 shows the shells of the investigated species.

Dry and alcohol preserved specimens of *L. erubescens* (N = 2) were obtained from the malacological collection of the Academy of Natural Sciences of Philadelphia (ANSP 128459 A9427H). Several specimens (frozen at –80ºC) of this species (N = 4), as well as of *L. undata* (N = 4) and *L. nivosa* (N = 5), were generously made available to us by P. Van Riel (Royal Belgian Institute of Natural Sciences, Brussels).

The specimens of each species, which were all adult, were dissected to remove the reproductive tract. Subsequently, the reproductive organs were drawn using a camera lucida. To

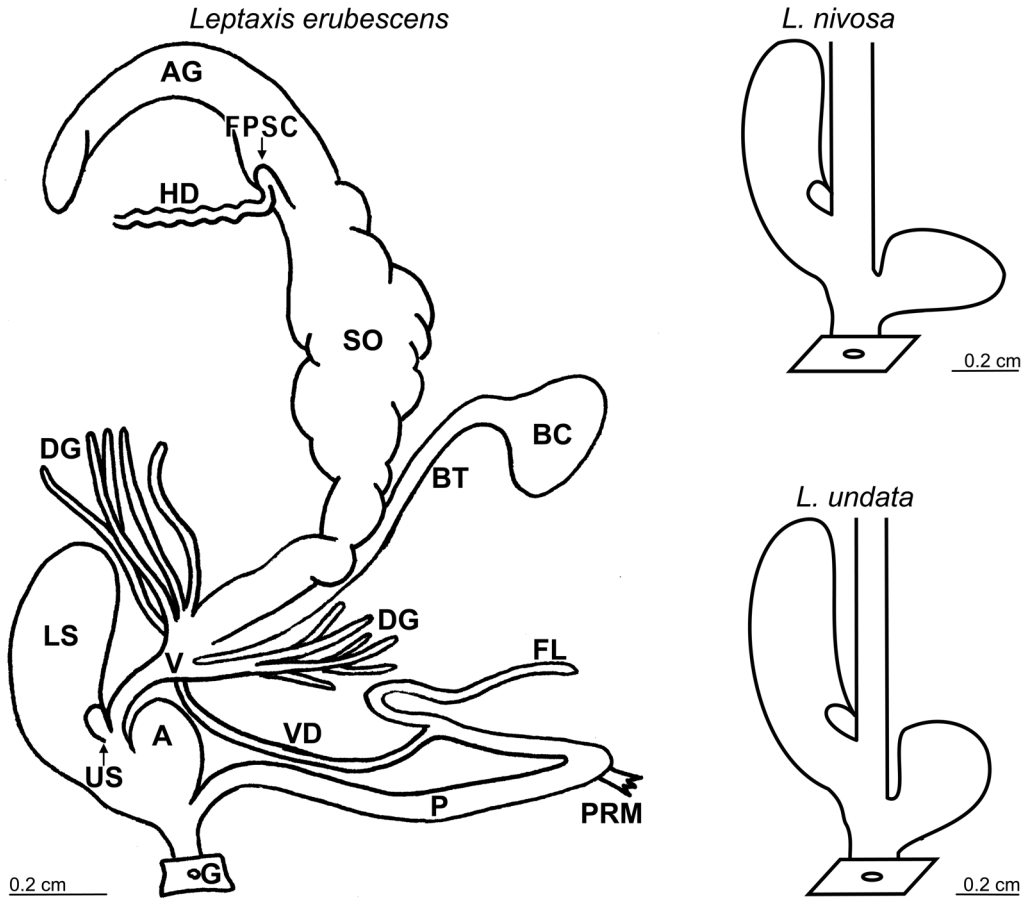

FIG. 2. Comparison of the position and relative size of the upper and lower stylophores of *Leptaxis erubescens*, *L. undata* , and *L. nivosa*. The reproductive system of *L. erubescens* is shown to depict the other reproductive structures that are mentioned in the text. Abbreviations: A, appendage; AG, albumen gland; BC, bursa copulatrix; BT, bursa tract; DG, digitiform gland; FL, flagellum; FPSC, fertilization pouch-spermathecal complex; G, genital pore; HD, hermaphroditic duct; LS, lower stylophore; P, penis; PRM, penis retractor muscle, SO, spermoviduct; US, upper stylophore; V, vaginal duct; VD, vas deferens.

avoid damage of the darts, the stylophores were carefully cut out of the reproductive tracts and placed overnight in 1N NaOH, which dissolved all the tissue and mucus but left the dart intact. For cross-sections darts were carefully broken in two. The intact and broken darts were consecutively prepared for electron microscopy by placing them on small aluminium plates with an electrically conducting adhesive (Leit-Tab, Plano). Subsequently, they were coated with gold using a Metalloplan (Leitz). The darts were then placed under a scanning electron microscope (S-530 SEM, Hitachi) and photos were taken.

RESULTS

In all the mature specimens of each species, one large stylophore, containing one dart, was present. This stylophore was positioned in such a way that it curved slightly around the vaginal duct. Besides this stylophore, we also found a small sac situated between the larger stylophore and the vaginal duct in all species. The position of this organ suggests that we are dealing with the rudiment of an upper stylophore. Additionally, a flattened, non-hollow appendage at the base of the vagina is present. Figure 2 gives an overview of the morphology of the investigated *Leptaxis* species showing the positions and relative sizes of the stylophores, the small sac (i.e., upper stylophore) and the appendage. The two mucus glands of each of the species are situated above the stylophore around the vaginal duct. Each of these digitiform glands has several branches that join at the base. These glands, as well as the rest of the reproductive system, are only depicted for *L. erubescens*.

The darts of all three species have a round base and a broad corona by which they are attached to a tubercle in the stylophore (Fig. 3).

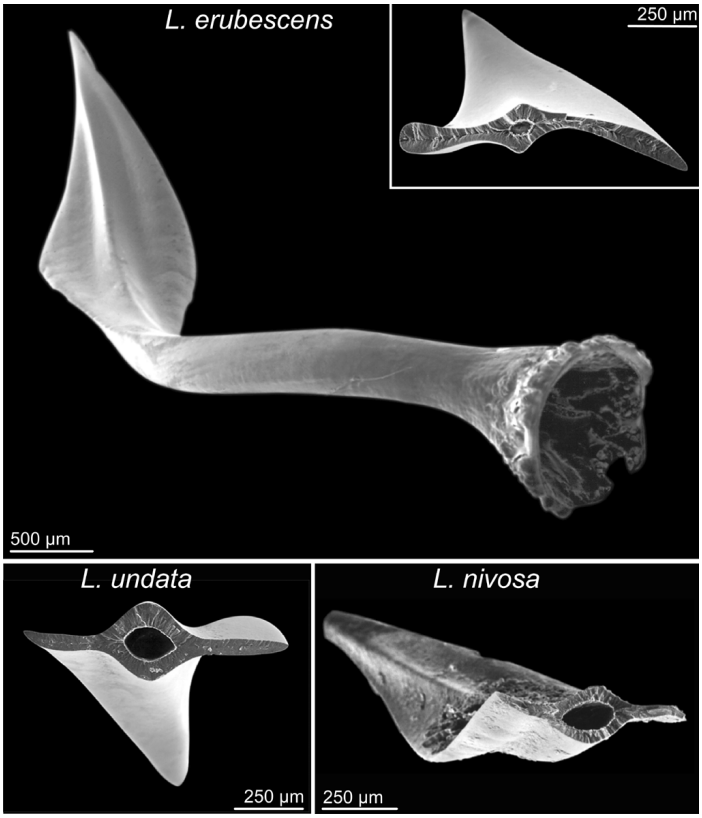

FIG. 3. Electron microscopic pictures of the darts of *Leptaxis erubescens*, *L. undata*, and *L. nivosa*.

Approximately halfway towards the tip of the dart the curved shaft broadens and flattens out, thus forming two large blades (Fig. 3). The dart is curved and lightly contorted, which is illustrated by the electron microscopic picture of the side view of a dart of *L. erubescens* (Fig. 3), also reflecting the shape of the stylophore.

DISCUSSION

It has long been thought, based on morphological data, that the genus *Leptaxis* fully conforms to the European Hygromiidae with one stylophore (Mandahl-Barth, 1943; Backhuys, 1975; Schileyko, 1989). Interestingly, Pilsbry (1894: 292–293) stated after having investigated several *Leptaxis* species: "I had expected to find in *Leptaxis* some archaic characters preserved; for its geographic position and the shell-peculiarities argue for the group an ancient origin; but the evidence shows that however remote in the past the type was derived from the continental fauna, the main anatomical features of modern European Helices were then well established". Our finding of the small organ just above the stylophore in the investigated species of *Leptaxis* suggests that Pilsbry was correct in expecting some ancient characters.

The position of the previously overlooked organ is consistent with the position of the upper stylophore in the genus *Trichia* (Schileyko, 1978a) and the internal or accessory dart sac in the genus *Hygromia* (Giusti & Manganelli, 1987; Nordsieck, 1987). Therefore, we conclude that the investigated species of the *Leptaxis* genus possess a rudiment of an upper stylophore. This rudiment has probably been overlooked for so long because the small organ is well hidden in connective tissue between the vaginal duct and the much larger functional stylophore that contains the contorted dart. Nevertheless, the presence of the upper stylophore in *Leptaxis* has important implications for the phylogenetic position of this genus within the Hygromiidae. Much of the molluscan phylogeny is heavily based on traits of the reproductive morphology and, especially within the Hygromiidae, the presence and number of (reduced) stylophores play an important role in the classification within the family (Nordsieck, 1987, 1993; Schileyko, 1989).

Several observations can be made with respect to the reproductive morphology of the family Hygromiidae. There are clear morphological differences between the phylogenetically older subfamily Trichiinae and the younger subfamily Hygromiinae. All Trichiinae have two pairs of stylophores, that is, two upper and two lower stylophores (e.g., *Trichia*: Schileyko, 1978a). Most of the differences in the stylophore morphology between genera of Trichiinae are relatively small, while important morphological changes are found within the Hygromiinae. In this subfamily, one pair of stylophores has been lost, consequently many species have one upper and one lower stylophore (e.g., *Hygromia*: Giusti & Manganelli, 1987). Additionally, a further reduction of the upper stylophore and an enlargement of the lower stylophore occurred (e.g., *Leptaxis*: this paper; *Lindholmomneme*: Schileyko, 1978b), culminating with total loss of the upper stylophore (e.g., *Monachoides*: Schileyko, 1978b, 1989).

Simultaneously with this evolution towards a single stylophore, the dart seems to become more elaborate. Perpendicular blades on the dart occur in several genera of Hygromiinae, resulting in different dart shapes, and increasing the dart's surface area. Presumably, this allows the dart to transfer larger amounts of the product from the mucus glands (Fedoseeva, 1994; Adamo & Chase, 1996; Koene & Schulenburg, submitted). However, it is still unclear whether the hygromiid dart is used in a similar way as the helicid dart (Koene & Chase, 1998a, b; Rogers & Chase, 2001; Landolfa et al., 2001) to transfer an allohormone (Koene & Ter Maat, 2001, 2002). Hence, behavioural data are required to determine how the *Leptaxis* dart is used. Observations of the mating behaviour of *Leptaxis* may also shed light on the function of the appendage at the base of the genital system (see also Mandahl-Barth, 1943).

To conclude, we found the rudiment of an upper stylophore in three species of *Leptaxis*, which has previously gone unnoticed (Mandahl-Barth, 1943; Backhuys, 1975, Schileyko, 1989). The presence of this small organ is of importance because it indicates that *Leptaxis* links Hygromiinae with two (upper and lower) stylophores (e.g., *Lindholmomneme*) and Hygromiinae with single stylophores (e.g., *Monachoides*). Therefore, our findings lead us to conclude that this genus is an intermediate form in the evolution towards a single stylophore.

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