DELAYED MATURATION IN THE GENUS *VESTIA* P. HESSE (GASTROPODA: PULMONATA: CLAUSILIIDAE): A MODEL FOR CLAUSILIID LIFECYCLE STRATEGY

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ABSTRACT

We studied the development of the reproductive system of two iteroparous land snails with determinate growth, *Vestia gulo* and *V. turgida*, in various stages of the life cycle: juvenile snails at five different stages, subadult snails during formation of their closing apparatus and adults. Maturation of the reproductive system is similar in both species but delayed in relation to the shell growth. Moreover, gonad development is faster than that of the remaining reproductive organs. The juvenile gonad contains only numerous dividing cells, gradually appearing spermatocytes and growing previtellogenic oocytes. Spermiogenesis starts during formation of the closing apparatus; 1 month after growth completion the number of mature spermatozoa packets, early vitellogenic and vitellogenic oocytes increases. Six months after growth completion, the gonad is histologically identical to that of reproducing individuals. During formation of the closing apparatus, the hermaphrodite duct becomes distinct as a slightly folded structure, the primordial spermatheca and mucous gland appear, and primordia of penis, oviduct and vagina as well as primordial albumen gland all become well visible. Three months after growth completion, all the organs are well developed and the reproductive system is morphologically mature. This is the time when the snails start to mate. Five to 6 months after growth completion, the snails are capable of retaining fertilized, developing eggs in their oviducts. Both clausiliids thus have a developmental strategy with a delay in maturation. This may decrease the time required to build the closing apparatus which reinforces the shell.

INTRODUCTION

Delayed onset of reproduction, observed in many plants, invertebrates and vertebrates, affects several life-cycle parameters (age-dependent mortality, fecundity etc.) and is a major topic in the study of the evolution of life histories (Koons, Metcalf & Tuljapurkar, 2008). It can evolve in situations in which advantages of delayed reproduction prevail over the disadvantage of decreased probability of reaching reproductive age. Such situations arise when a prolonged prereproductive phase enables the individual to continue growing, thus increasing the initial fecundity or the quality of offspring (e.g. because of improved parental care or higher social status). Delayed maturation can be considered in terms of intra-population variation or as a component of the species' biology. It remains debatable under which conditions (stable versus unstable environment) the delay may be of advantage (Tuljapurkar, 1990; Wilbur & Rudolf, 2006; Koons *et al.*, 2008). In this paper, we present the growth and maturation schedule in two long-lived, iteroparous terrestrial pulmonate snails of the family Clausiliidae. Knowledge of developmental strategies, including the delay of maturation, may provide invaluable data for understanding the evolution of life histories.

The Clausiliidae include *ca.* 1300 recent species and approximately 450 of these inhabit Europe (Nordsieck, 1978, 2007). Their life cycles are rather poorly known and information is available for only 27 European species (e.g. Steenberg, 1914; Frömming, 1954; Likharev, 1962; Nordsieck, 1966, 1985, 2005, 2007; Piechocki, 1982; Baur, 1990; Baur & Baur, 1992;

Schilthuizen & Lombaerts, 1994; Wirth, Baur & Baur, 1997; Kuźnik-Kowalska, 1998; Giokas & Mylonas, 2002; Maltz & Sulikowska-Drozd, 2008; Páll-Gergely & Németh, 2008; Maltz & Pokryszko, 2009; Sulikowska-Drozd, 2008, 2009a; Tlachač, 2008). With few exceptions, the literature contains no detailed data on clausiliid maturation. Likharev (1962) argues that clausiliids are protandrous, albeit with no evidence. More recent reports on life cycles of several Mediterranean members of the genus *Albinaria* (Schilthuizen & Lombaerts, 1994; Giokas & Mylonas, 2002) suggest that growth termination in clausiliids does not imply sexual maturity, since there is a considerable time-lag between reaching ultimate size and the beginning of reproduction. These authors point to the possible relation of delayed maturation with the seasonality of the Mediterranean climate (aestivation during dry season), but this conjecture has not been confirmed at the anatomical and histological level of the reproductive system. Delayed maturity has been observed in 14 northern European clausiliid species (Maltz & Sulikowska-Drozd, 2008); kept under constant laboratory conditions, individuals produced eggs/offspring at the earliest 5–8 months after growth is completed.

Life cycles of the two species selected for study, *Vestia gulo* and *V. turgida*, have been well studied in the laboratory and in the field (Sulikowska-Drozd, 2009a). Although congeneric, they show slightly different reproductive strategies. *Vestia gulo* shows a short egg retention while *V. turgida* is ovoviviparous (Sulikowska-Drozd, 2009a). In the laboratory, shell growth in both species takes 3.5–4 months and terminates with the formation of a closing apparatus and reflexed lip (Maltz & Sulikowska-Drozd, 2008). Both species are long-lived and

iteroparous. In the laboratory, they reproduce during four consecutive years and adults marked in the field survive for at least 4 years (A. Sulikowska-Drozd, unpubl.). The above-mentioned observations have encouraged us to undertake further, more detailed studies on the maturation and development of the reproductive system, with special reference to gonad activity, in order to describe clausiliid developmental strategies.

MATERIAL AND METHODS

Development of the reproductive system was studied from spring 2007 until autumn 2008. Specimens of *Vestia gulo* and *V. turgida* originated from laboratory cultures. Snails from Krościenko (Pieniny Mountains, 425 m a.s.l.) constituted the stock material for the laboratory cultures. Juveniles were kept at 20–24°C, with natural photoperiod. Date of hatching, growth rate and date of reaching maximal size were recorded for each individual. Animals were fed with lettuce, and humidity in the containers was kept high and constant. Snails were given access to a calcium source. Initially, the following development stages were dissected: juvenile (J III: 3.0–3.9 whorls; J IV: 4.0–4.9 whorls; J V: 5.0–5.9 whorls; J VI: 6.0–6.9 whorls; J VII: 7.0–7.9 whorls; J VIII: 8.0–8.9 whorls; J IX: 9.0 whorls till the beginning of closing apparatus formation); subadult (SA: stage during the formation of the closing

apparatus and lip) and conchologically adult (AM1: 1 month after lip completion; AM3: 3 months after lip completion; AM6: 6 months after lip completion). Stage J V was the earliest in which the gonad could be located and dissected. Consequently, the analysed stages included J V to AM6. The total number of individuals examined was 275.

Anatomical studies

The reproductive systems were obtained from animals killed in boiling water. Shells were dissolved in 0.1 M HCl (Nordsieck, 2007) and the gonad was dissected together with the surrounding hepatopancreas (in the Clausiliidae, the gonad is deeply embedded in the digestive gland). Reproductive systems were preserved in 75% ethanol. Five specimens of each juvenile stage, and 10 specimens of each of the stages SA, AM1, AM3 and AM6 were dissected for each species (total 130 specimens). The presence/absence of individual organs, and their stage of development (primordial or developed organ), compared to mature, reproducing specimens, were noted.

Histological studies

The gonads with adjacent hepatopancreas fragments were dissected and fixed in Bouin's fluid or in 4%, and then 10%, buffered formaldehyde solution. The material was dehydrated in a
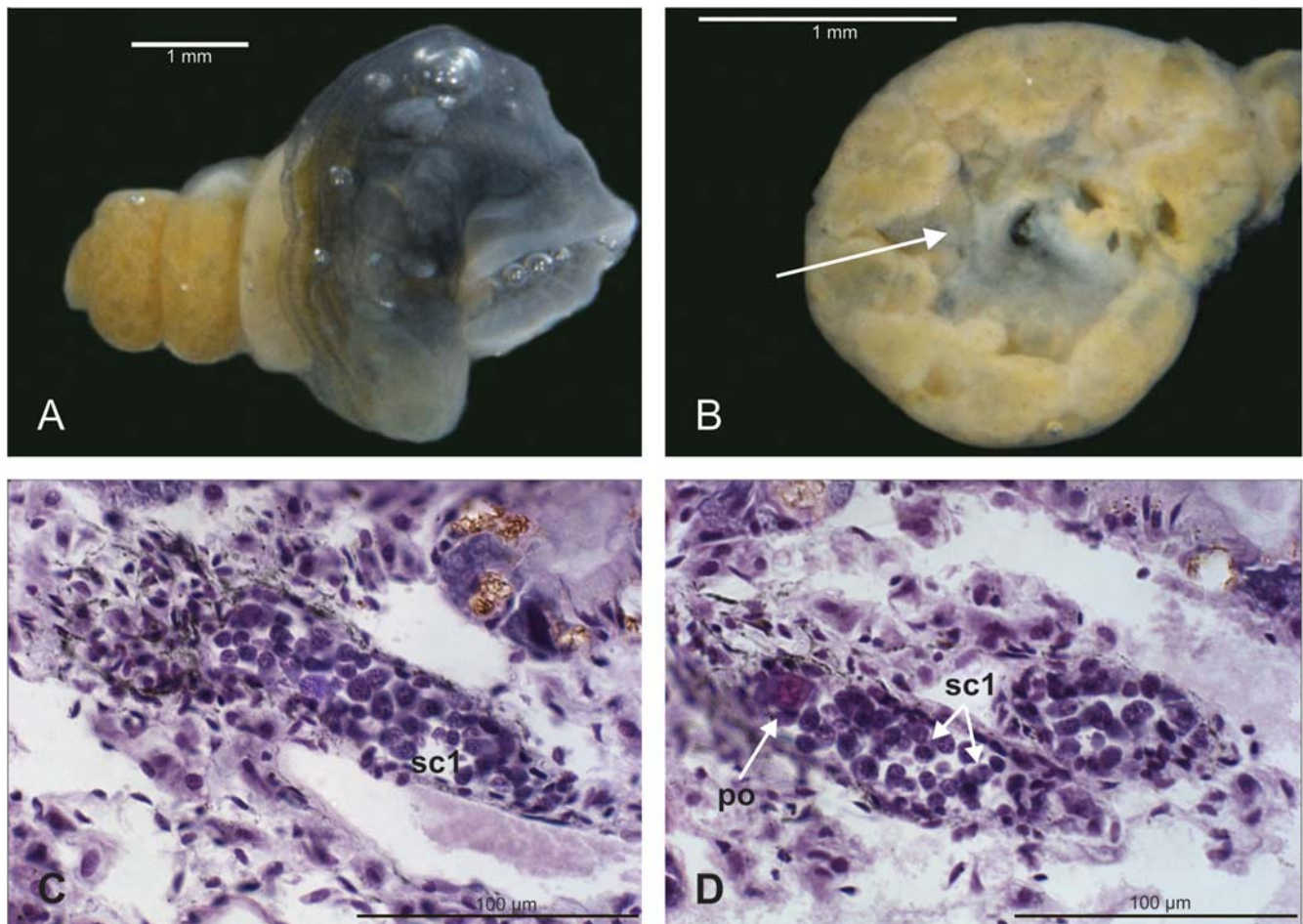

Figure 1. Hermaphroditic gland of *Vestia*. Stage J VI. **A**. Hepatopancreas with gonad invisible from outside (*V. gulo*). **B**. Lower whorl of hepatopancreas with visible gonad (*V. gulo*). **C**. Section through the gonad (*V. gulo*). **D**. Section through the gonad (*V. turgida*). Abbreviations: po, growing previtellogenic oocytes; sc1, spermatocytes I in meiotic prophase I. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

graded ethanol series and xylene, and embedded in paraffin (Paraplast Plus with 8% DMSO). A rotational microtome was used to make several 7-μm sections. Following rehydration, slides were stained with Delafield haematoxylin and 25% aqueous solution of eosin (Zawistowski, 1986), dehydrated, cleared and cover slipped with DPX mounting medium for light-microscopical observations (Olympus BHS). For each species, 8–10 gonads of each developmental stage (total of 145 specimens) were examined.

The classification of male and female germ-line cells were based on the literature (Noyce, 1973, after Tompa, 1984; Kubrakiewicz, 1985; Griffond & Bolzoni-Sungur, 1986; Rodrigues *et al.*, 1998; Healy, 2001; Maltz, 2003a). Five stages of spermatogenesis were identified: sc1, spermatocytes I in meiotic prophase I (sphaeroidal cell, ca. 8–10 μm in diameter, nucleus large in relation to the small quantity of cytoplasm); sc2, spermatocytes II (ca. 11–17 μm in diameter, with a more abundant cytoplasm, rosette-forming); st1, early spermatids (small cells, ca. 8–10 μm in diameter, sphaeroidal in shape); st2, late spermatids with thick flagella; s, mature spermatozoa forming packets. Four stages of development were distinguished during oogenesis: po, growing previtellogenic oocytes (ranging from spherical to oval, up to ca. 30 μm diameter, no visible granules in cytoplasm); ev, early vitellogenic oocytes, cells in initial phase of vitellogenesis (ranging from oval to elongate, up to ca. 80–90 μm diameter; granules appear in cytoplasm); vo, vitellogenic oocytes (elongated to rounded,

ca. 90–120 μm diameter; increasing number of granules in cytoplasm); mo, mature oocytes (large, rounded cells, of more than 120 μm diameter, most often 125–135, cytoplasm filled with numerous cytoplasmic granules). Cell diameter was measured with a calibrated eye-piece on a microscope (WF DIN 10×).

RESULTS

Anatomically and histologically, the rate and pattern of the reproductive system development were similar in *Vestia gulo* and *V. turgida*; the description below pertains to both species, with differences indicated when necessary. Gonad development is considerably faster, compared to that of the other reproductive organs, hence the results are divided in two parts: (1) changes in the gonad morphology in various development stages in the context of the histological images; (2) development of the remaining reproductive organs.

Gonad development

The gonad of adult, sexually mature individuals is composed of a few lobes (most often four to five), each divided into acini (mostly six to nine) and efferent duct; the gonad is located in the first, widest whorl of the hepatopancreas. The efferent ducts, which merge to form the hermaphrodite canal, run on the columellar side, while the gonad lobes are radially
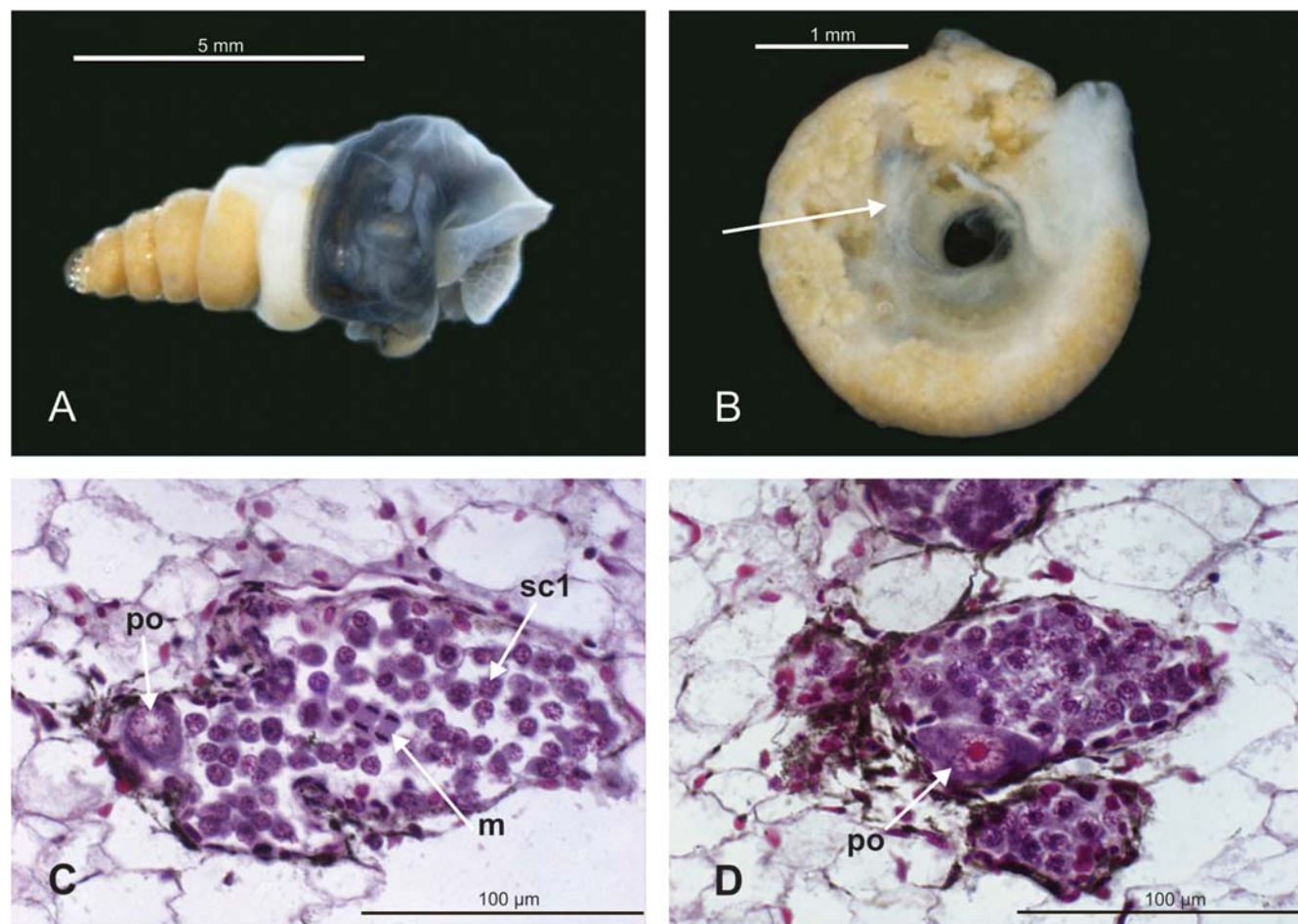

Figure 2. Hermaphroditic gland of *Vestia*. Stage J VII. **A**. Hepatopancreas with gonad invisible from outside (*V. gulo*). **B**. Lower whorl of hepatopancreas with visible gonad (*V. gulo*). **C**. Section through the gonad (*V. gulo*). **D**. Section through the gonad (*V. turgida*). Abbreviations: m, mitoses; po, growing previtellogenic oocytes; sc1, spermatocytes I in meiotic prophase I. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

arranged in the hepatopancreas, extending from the canal to the shell wall. The population of male (spermatogonia, spermatocytes, spermatids, spermatozoa) and female (oocytes at various developmental stages) germ cells are distinguishable in histological slides of the acini.

The structure and histological image of the gonad of juvenile snails and individuals which have just terminated growth differed considerably from those found in sexually mature specimens. Consecutive developmental stages of the gonad are shown in Figures 1–7, and the proportion of gonads of each stage with the presence of various types of germ cells is presented in Figure 8.

J V (1.5–2 months) is the earliest stage where the gonad primordia can be identified in the hepatopancreas. The gonad lobes occupy only the columellar part of the digestive gland and are not divided into acini. They are very difficult to distinguish anatomically. Histologically, only small groups of small cells with nuclei occupying almost all the cell volume can be seen. These are most probably groups of spermatogonia or spermatocytes I in meiotic prophase I and oogonia or small previtellogenic oocytes I.

At stage J VI (ca. 2 months) (Fig. 1A, B) identification of the gonad primordia is much easier. The gonad lobes reach half the hepatopancreas width but are still not divided into acini. They contain numerous germ cells – spermatogonia and groups of early spermatocytes I in the meiotic prophase I, filling the entire gonad volume (Fig. 1C, D). The cells located near the walls of the acini are most probably small previtellogenic oocytes in prophase I. Only one gonad of *V. turgida* contained single, growing previtellogenic oocytes (6.25% of all examined specimens of that stage) (Figs 1D, 8).

At stage J VII (ca. 2.5 months) (×Fig. 2A, B), the appearance and histology of the gonad (Fig. 2C, D) are very similar to the preceding stage. Only the volume of the gonad lobes increases somewhat because of the progressing cell divisions (Fig. 2C). Growing previtellogenic oocytes were observed in 90% of the examined gonads (*V. turgida* 100%, *V. gulo* 80%) (Fig. 8).

At stage J VIII (ca. 3 months) (×Fig. 3A, B), the gonad lobes occupy a greater volume, almost three quarters of the distance between the columella and shell walls, penetrating the parenchyma of the hepatopancreas (Fig. 3B). Their acini become gradually differentiated. Besides the active dividing germ cells, increasingly frequent growing previtellogenic oocytes, located close to the acinar walls, are visible (Figs 3C, D, 8).

At stage J IX (ca. 3.5 months) (×Fig. 4A, B), the acini become more distinct, with their tips increasingly pigmented. Besides the dividing cells, the first spermatocyte rosettes, growing previtellogenic and the first early vitellogenic oocytes are visible in 33% of the examined gonads (*V. turgida* 40%, *V. gulo* 25%) (Figs 4C, D, 8).

During the formation of the closing apparatus and lip (SA) (3.5–4 months), the gonad lobes extend through the entire
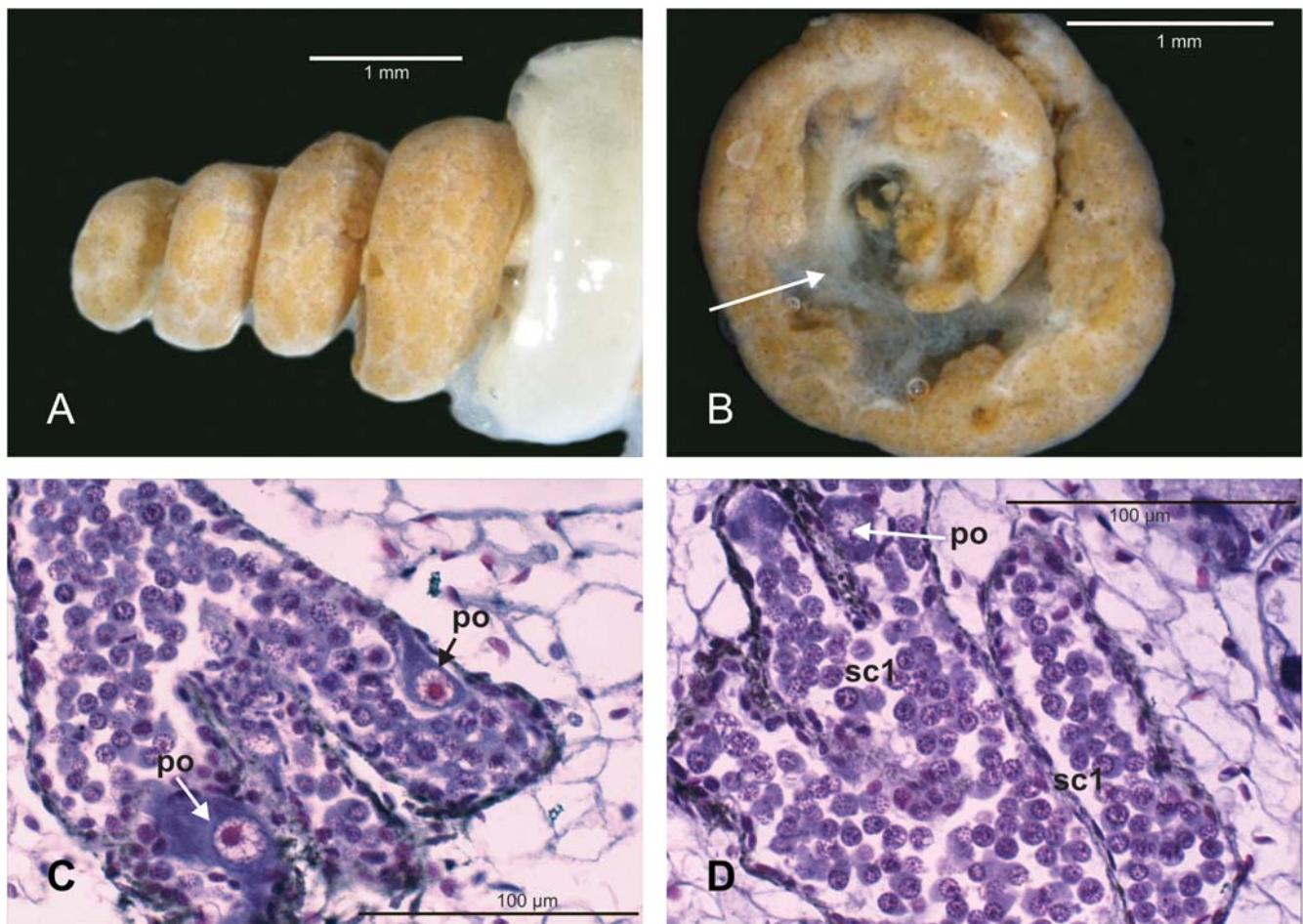
Figure 3. Hermaphroditic gland of *Vestia*. Stage J VIII. **A**. Hepatopancreas with gonad invisible from outside (*V. gulo*). **B**. Lower whorl of hepatopancreas with visible gonad (*V. gulo*). **C, D**. Section through the gonad (*V. gulo*). Abbreviations: po, growing previtellogenic oocytes; sc1, spermatocytes I in meiotic prophase I. This figure appears in colour in the online version of *Journal of Molluscan Studies*.
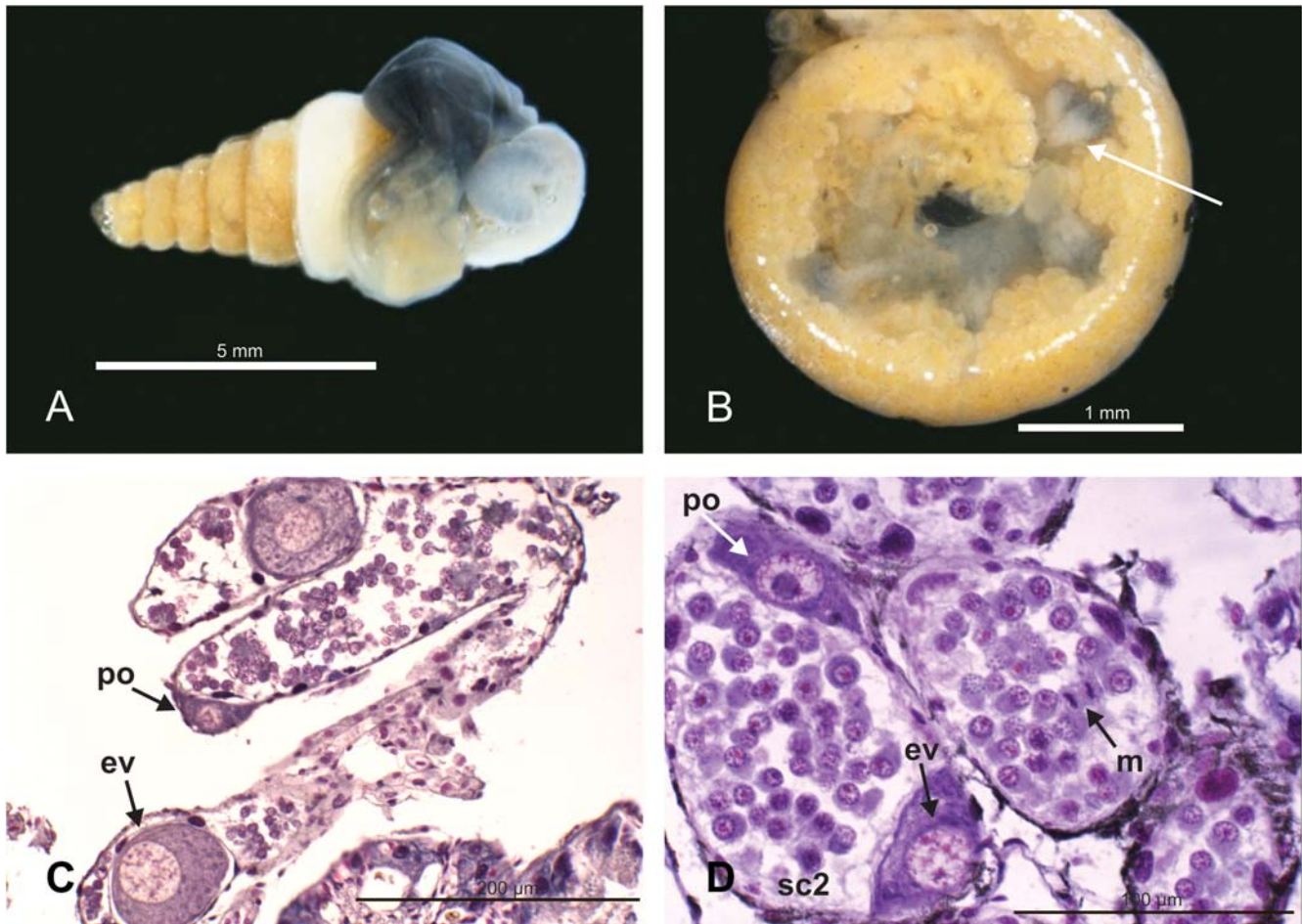
Figure 4. Hermaphroditic gland of *Vestia*. Stage J IX. **A**. Hepatopancreas with gonad invisible from outside (*V. gulo*). **B**. Lower whorl of hepatopancreas with gonad; gonad lobes with emerging acini (*V. gulo*). **C**. Section through the gonad (*V. turgida*). **D**. Section through the gonad (*V. gulo*). Abbreviations: ev, early vitellogenic oocytes; m, mitoses; po, growing previtellogenic oocytes; sc1, spermatocytes I in meiotic prophase I; sc2, rosette-forming spermatocytes II. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

width of the hepatopancreas, from the columella to the shell walls (×Fig. 5A, B), and become visible through the translucent shell. The acini, with their tips black pigmented, are distinct (Fig. 5B). They are filled with numerous cells at various stages of division, the number of rosette-forming spermatocytes increases and the first spermatids at various development stages (spermiogenesis) and spermatozoa appear (Fig. 5C–F). The first packets of mature spermatozoa appeared in two gonads of *V. turgida* (10% of all examined specimens of that stage) (Figs 5E, 8).

One month after lip completion (AM1), the gonad is fully formed (×Fig. 6A, B). It still contains numerous dividing cells but the number of mature packets of spermatozoa increases, as well as the number of growing previtellogenic and early vitellogenic oocytes (Figs 6C, D, 8). Three months after growth completion (AM3), the acini (Fig. 6E) mostly contain numerous packets of spermatozoa, and the cells in meiotic prophase become fewer. Together with an increasing number of growing previtellogenic and early vitellogenic oocytes, the first vitellogenic oocytes appear (27.7% of examined gonads: *V. turgida* 37.5%, *V. gulo* 20%) (Figs 6F, 8).

The gonad of individuals 6 months after growth completion (AM6) (Fig. 7A–F) is identical with that of mature individuals of two or more years. Besides the mitotically dividing cells and the cells in meiotic prophase, which are distinctly less numerous, there are spermatocyte rosettes, spermatids,

maturing and mature spermatocytes and growing previtellogenic oocytes (each type observed in all gonads), vitellogenic oocytes (81.25% of the examined gonads) and the first mature oocytes (50% of the examined gonads) in the gonad, indicating maturity (Fig. 8).

Development of the remaining reproductive organs

The development of the remaining reproductive organs is shown diagrammatically in Figure 9, and selected organs and changes in their structure are shown in Figures 10 and 11. The development of male and female organs is synchronous.

At stages J VI–J IX, the reproductive system consists of thin, translucent threads of primordia of the hermaphrodite canal, spermoviduct, epiphallus, penis, oviduct and vagina (Fig. 9). During the formation of the closing apparatus and lip (SA), primordia of most organs can be seen: the hermaphrodite canal in the form of a slightly folded structure passing into spermoviduct (a rather wide, unfolded duct) through the characteristically bent primordial carrefour; the primordial spermatheca also becomes distinct (it reaches two-thirds of its ultimate length), with a delicately marked spherical swelling at the end; the primordial mucous gland is a translucent, unfolded tube, attached on its entire length to the oviduct and lower spermoviduct; the penis primordium becomes clearly visible (a distinct swelling), as well as primordia of the oviduct
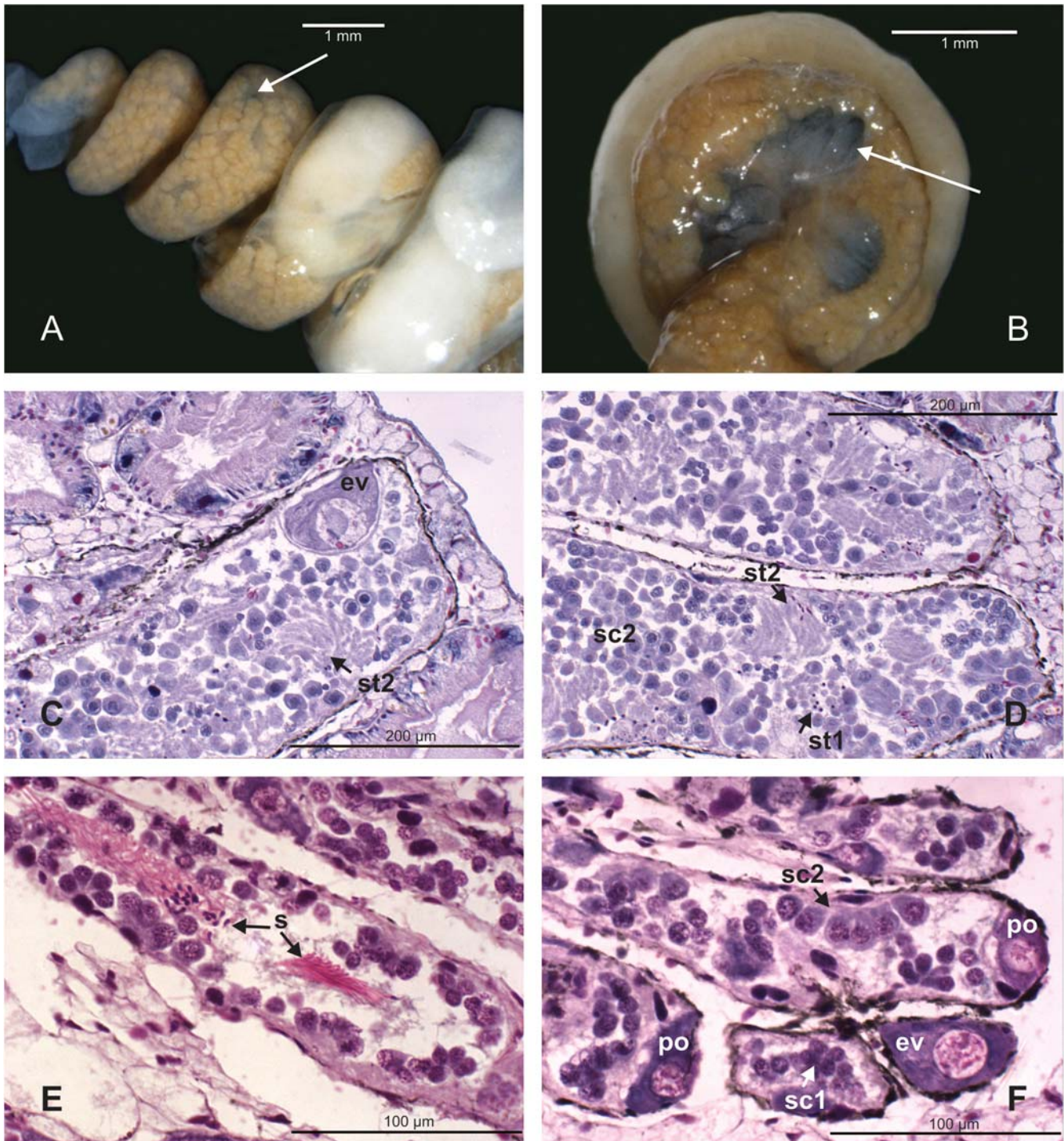
Figure 5. Hermaphroditic gland of *Vestia*. Stage SA. **A**. Hepatopancreas with visible gonad (*V. gulo*). **B**. Lower whorl of hepatopancreas with gonad, lobes clearly divided into acini (*V. gulo*). **C, D**. Section through the gonad (*V. gulo*). **E, F**. Section through the gonad (*V. turgida*). Abbreviations: ev, early vitellogenic oocytes; po, growing previtellogenic oocytes; s, spermatozoa; sc1, spermatocytes I; sc2, rosette-forming spermatocytes II; st1, early spermatids; st2, late spermatids. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

and vagina. The division into these two sections is possible due to the presence of the primordial spermatheca and mucous gland. Sometimes at this stage a primordial albumen gland is also visible (Figs 9, 10A, B).

One month after growth termination, the spermatheca becomes thicker, the mucous gland is clearly visible and folded, the penis has a distinct swelling on the border with the epiphallus, and on the surface of the oviduct a strongly pigmented epithelium appears, forming transverse grooves. The hermaphrodite duct becomes strongly folded (Figs 9, 10C, D). Three months after growth termination, all the reproductive organs are well developed and the reproductive system assumes its final form (Figs 9, 11A, B), while 6 months after growth completion (Figs 9, 11C, D), fertilized eggs with developing embryos can be found in the oviduct.
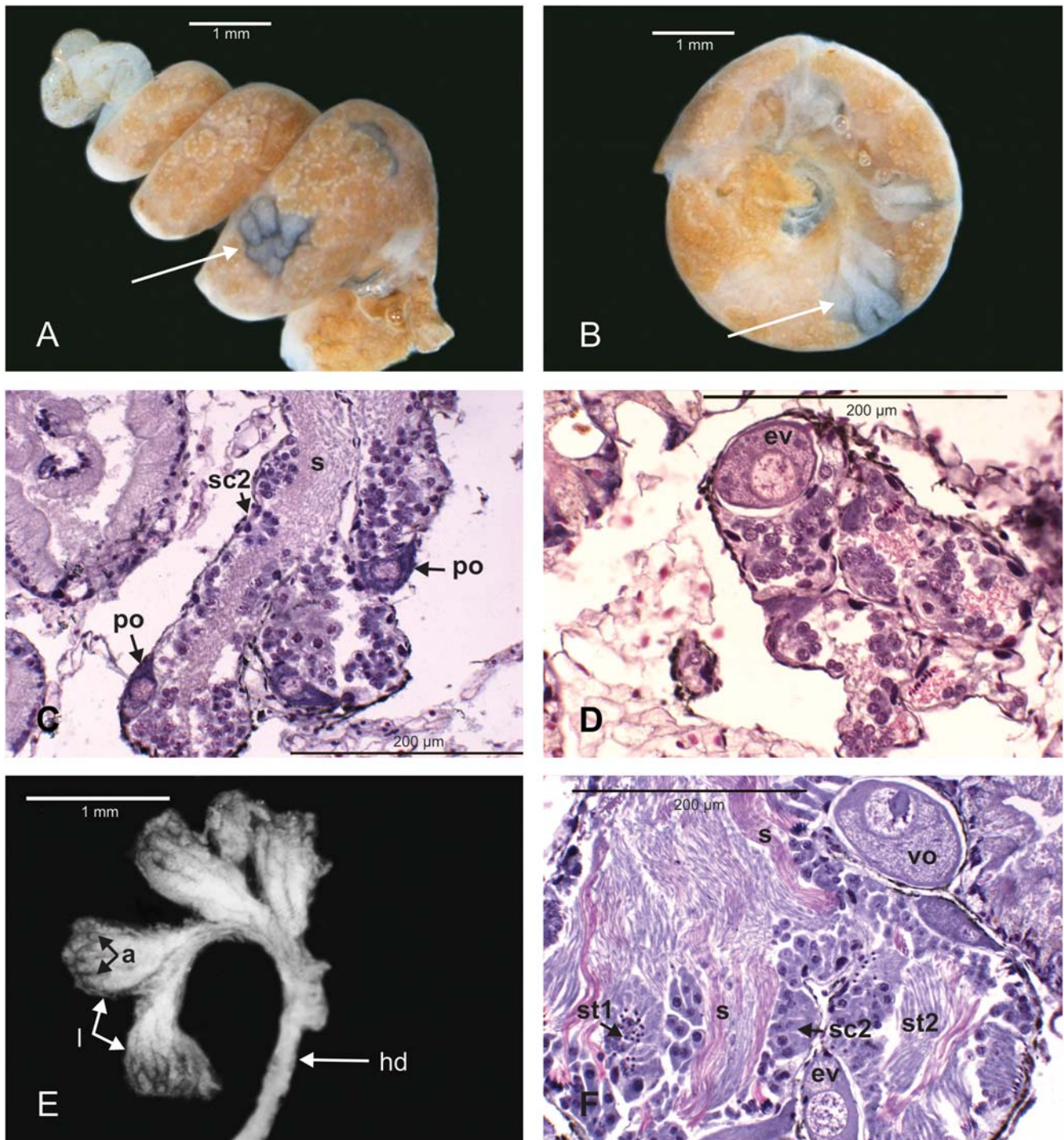
Figure 6. Hermaphroditic gland of *Vestia*. **A**. Stage A M1; hepatopancreas with visible gonad (*V. gulo*). **B**. Stage A M1; lower whorl of hepatopancreas with gonad (*V. gulo*). **C**. Stage A M1; section through the gonad (*V. gulo*). **D**. Stage A M1; section through the gonad (*V. turgida*). **E**. Stage A M3; prepared gonad (*V. gulo*). **F**. Stage A M3; section through the gonad (*V. gulo*). Abbreviations: a, acini; ev, early vitellogenic oocytes; hd, hermaphroditic duct; l, lobes; m, mitoses; po, growing previtellogenic oocytes; s, spermatozoa; sc2, rosette-forming spermatocytes II; st1, early spermatids; st2, late spermatids; vo, vitellogenic oocytes. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

DISCUSSION

Development strategies

With respect to life-history strategies, land snails can be divided into semelparous species that reproduce during one season and die, and iteroparous species that reproduce repeatedly in consecutive seasons/years (Heller, 2001). In this paper, we analysed other important lifecycle parameters such as the growth schedule and the onset of reproduction.

Terrestrial pulmonate snails can be divided into species with indeterminate shell growth and determinate shell growth. In the first group (e.g. Succineidae, Endodontidae, Vitrinidae,
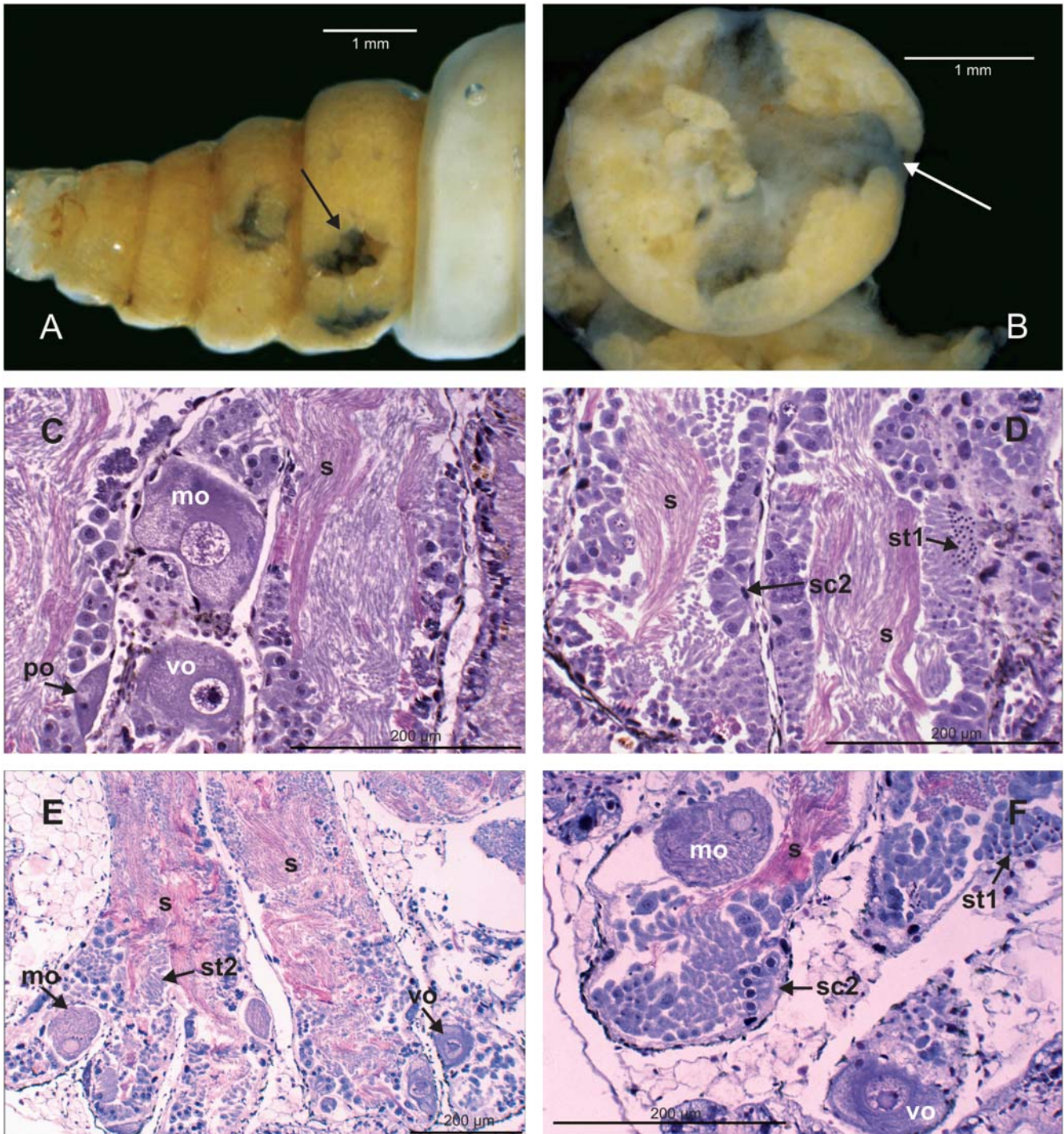
Figure 7. Hermaphroditic gland of *Vestia*. Stage A M6. **A**. Hepatopancreas with visible gonad (*V. gulo*). **B**. Lower whorl of hepatopancreas with gonad (*V. gulo*). **C, D**. Section through the gonad (*V. gulo*). **E, F**. Section through the gonad (*V. turgida*). Abbreviations: mo, mature oocytes; po, growing previtellogenic oocytes; s, spermatozoa; sc2, rosette-forming spermatocytes II; st1, early spermatids; st2, late spermatids; vo, vitellogenic oocytes. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

Zonitidae), signs of maturity are a considerable growth deceleration (since part of the energy is invested in reproduction), the occurrence of mating behaviour and the production of offspring. This has been observed, for example, in *Punctum pygmaeum* (Baur, 1987) and species of the genera *Succinea* (Jackiewicz, 1980, 2003) and *Discus* (Kuźnik-Kowalska, 2006). Individuals of these species with a complete reproductive system continue to grow (e.g. Umiński, 1975; Jackiewicz &

Zboralska, 1994; Jackiewicz, 2003). Thus, species with indeterminate shell growth have a developmental strategy of parallel growth and maturation, followed by reproduction and further growth.

The second group of species shows determinate growth. The shell is provided with a reflexed, thickened lip and often very complex structures in the aperture (e.g. teeth, lamellae, folds). Formation of such structures is a morphological sign of growth
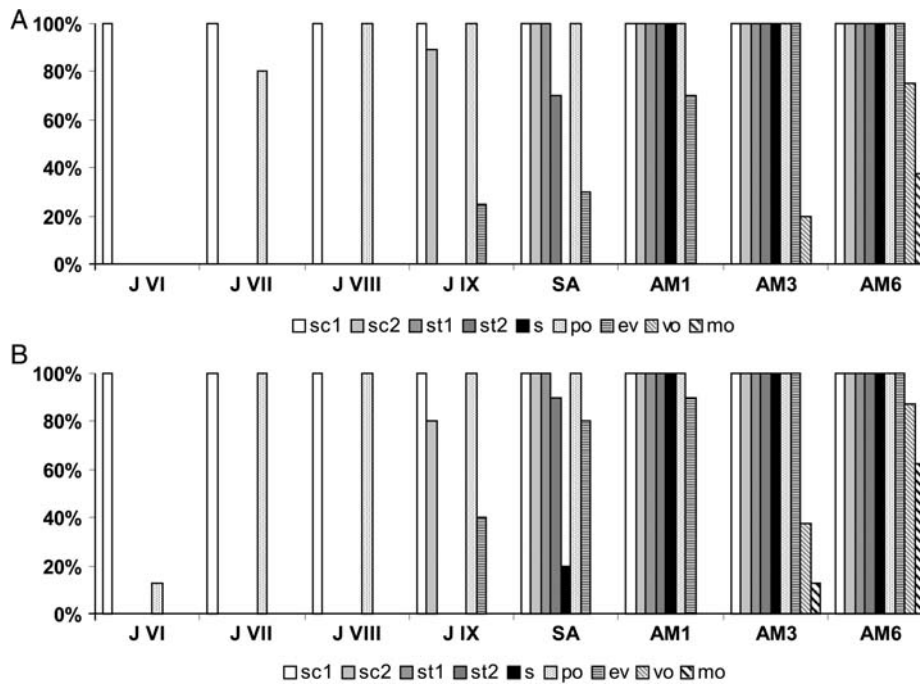
Figure 8. Gonad activity in ontogeny of *Vestia* (stages: J VI–A M6). Percentage of gonads of each stage, particular kinds of male and female germ cells found in. **A**. *V. gulo*. **B**. *V. turgida*. Abbreviations: ev, early vitellogenic oocytes; mo, mature oocytes; po, growing previtellogenic oocytes; s, spermatozoa; sc 1, spermatocytes I in meiotic prophase I; sc2, rosette-forming spermatocytes II; st1, early spermatids; st2, late spermatids; vo, vitellogenic oocytes.
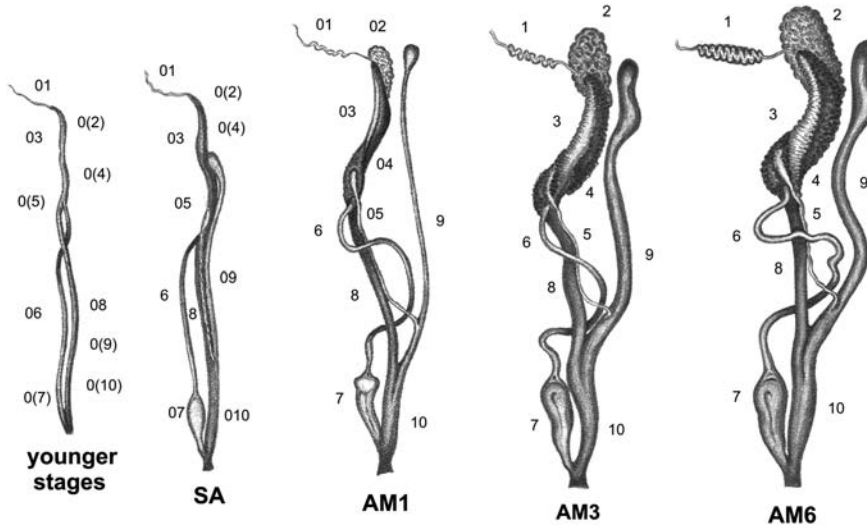

Figure 9. Development of reproductive system in *Vestia*. Semi-diagrammatic. Abbreviations: 1, hermaphroditic duct; 2, albumen gland; 3, spermoviduct; 4, prostate; 5, mucus gland; 6, vas deferens; 7, penis; 8, free oviduct; 9, bursa copulatrix; 10, vagina; 0(X), organ absent; OX, primordial organ; X, developed organ. For definition of growth stages, see text.

completion. This group includes, for example, representatives of the families Cochlicopidae, Vertiginidae, Orculidae, Chondrinidae, Pupillidae, Valloniidae, Enidae, Clausiliidae, Bradybaenidae and Helicidae. It is commonly assumed that attainment of final size is an indication of sexual maturity (Likharev, 1962; Baur, 1984 and references therein). This has been confirmed by detailed studies on *Vertigo pusilla*, *Helicodonta obvoluta* and *Helix lutescens* (Pokryszko, 1990; Koralewska-Batura, 1994, 1999; Maltz, 2003a, b; Mazurkiewicz & Pokryszko, 2005). It also has been observed that in Helicidae,

the reproductive system can be fully developed prior to, or during, lip formation (Koralewska-Batura, 1994; Maltz, 2003a). In addition, conchologically subadult *Monacha cantiana* (Chatfield, 1968), *Theba pisana*, *Helix pomatia* (Cowie, 1980) and *Arianta arbustorum* (Baur, 1984) have been observed to reproduce before completion of shell growth. Thus, these species represent another developmental strategy, with parallel growth and maturation followed by reproduction and no growth.

In the Clausiliidae, which have determinate shell growth, the situation is different. Field and laboratory observations
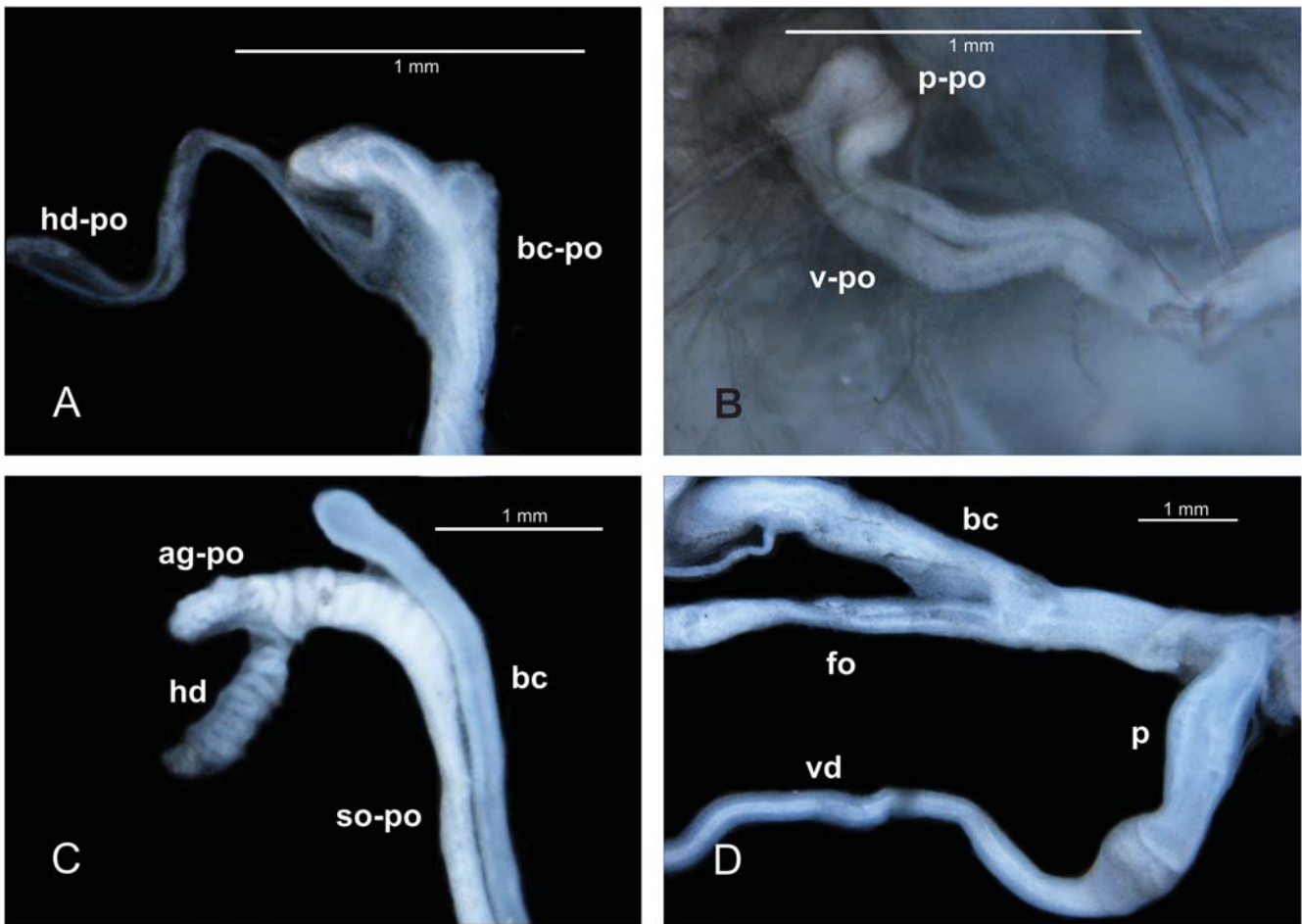
Figure 10. *Vestia gulo*. Reproductive system development, showing selected important parts. **A**, **B**. Stage SA. **C**, **D**. Stage A M1. Abbreviations: ag, albumen gland; bc, bursa copulatrix; fo, free oviduct; hd, hermaphroditic duct; p, penis; po, primordial organ; so, spermoviduct; v, vagina; vd, vas deferens. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

have shown that there is a considerable time-lag between the moment of reaching final shell size and the moment of first reproduction. In the laboratory, in pairs, first reproduction takes place only 5–8 months after growth completion in *Vestia gulo* and *V. turgida*. A similar situation is found in *Cochlodina laminata*, *Charpentieria ornata*, *Macrogastra ventricosa*, *M. latestriata*, *M. tumida*, *Clausilia parvula*, *Laciniaria plicata*, *Alinda biplicata*, *Balea stabilis* and *Vestia elata* (Maltz & Sulikowska-Drozd, 2008; Sulikowska-Drozd, 2008). The same has been observed in field studies on Greek representatives of *Albinaria* (Schilthuizen & Lombaerts, 1994; Giokas & Mylonas, 2002). Upon growth completion (SA), studied members of Clausiliidae still do not have a fully developed reproductive system (Maltz & Sulikowska-Drozd, 2008). The rapid development of the reproductive system takes place during a few months after reaching final shell size. Maturation of the gonad is also attained much later (i.e. between the third and sixth months after growth termination) than in non-clausiliid species with determinate shell growth such as *V. pusilla* and *H. obvoluta* (Maltz, 2003a; Mazurkiewicz & Pokryszko, 2005), in which the gonad during formation of apertural structures is histologically identical with that of mature adults. Hence, clausiliids represent yet another development strategy, with delayed sexual maturation and reproduction several months after shell growth has ceased.

This reproductive strategy may only apply to clausiliids with a complex closing apparatus. In the laboratory, and under high-density conditions, *Balea perversa* has been observed to reproduce 1–2 months after attaining ultimate size, with slightly further growth following first reproduction (Baur, 1990; Baur & Baur, 1992), which places its development strategy close to that of Succineidae, Endodontidae or Vitrinidae, rather than to Helicidae or Clausiliidae. There is no information on the reproductive system development in *B. perversa*. The species has reduced apertural structures (Nordsieck, 1982, 2007). As a result the adult shell resembles late juvenile stages of clausiliids which have preserved their closing apparatus. This hints at a possible case of neoteny, but this hypothesis requires more detailed studies.

The earlier growth and later maturation may be associated with the presence of a closing apparatus and the change of shell shape during its formation. The function of apertural structures and their advantages and disadvantages have been widely discussed in several pulmonates (Pokryszko, 1997 and references therein). In clausiliids, the characteristic and essential component of apertural barriers is the clausilium, a lamella transformed into a 'door' (Nordsieck, 1982, 2007) that protects the retracted animal from desiccation and/or predators. The role of the remaining components (folds, lamellae) probably includes stabilization of the adult shell during locomotion (a narrow, spindle-shaped shell is proportionally large compared to the body), protection of pallial organs against the pressure from neighbouring organs (e.g. alimentary canal) and division of the mantle cavity into the respiratory and excretion
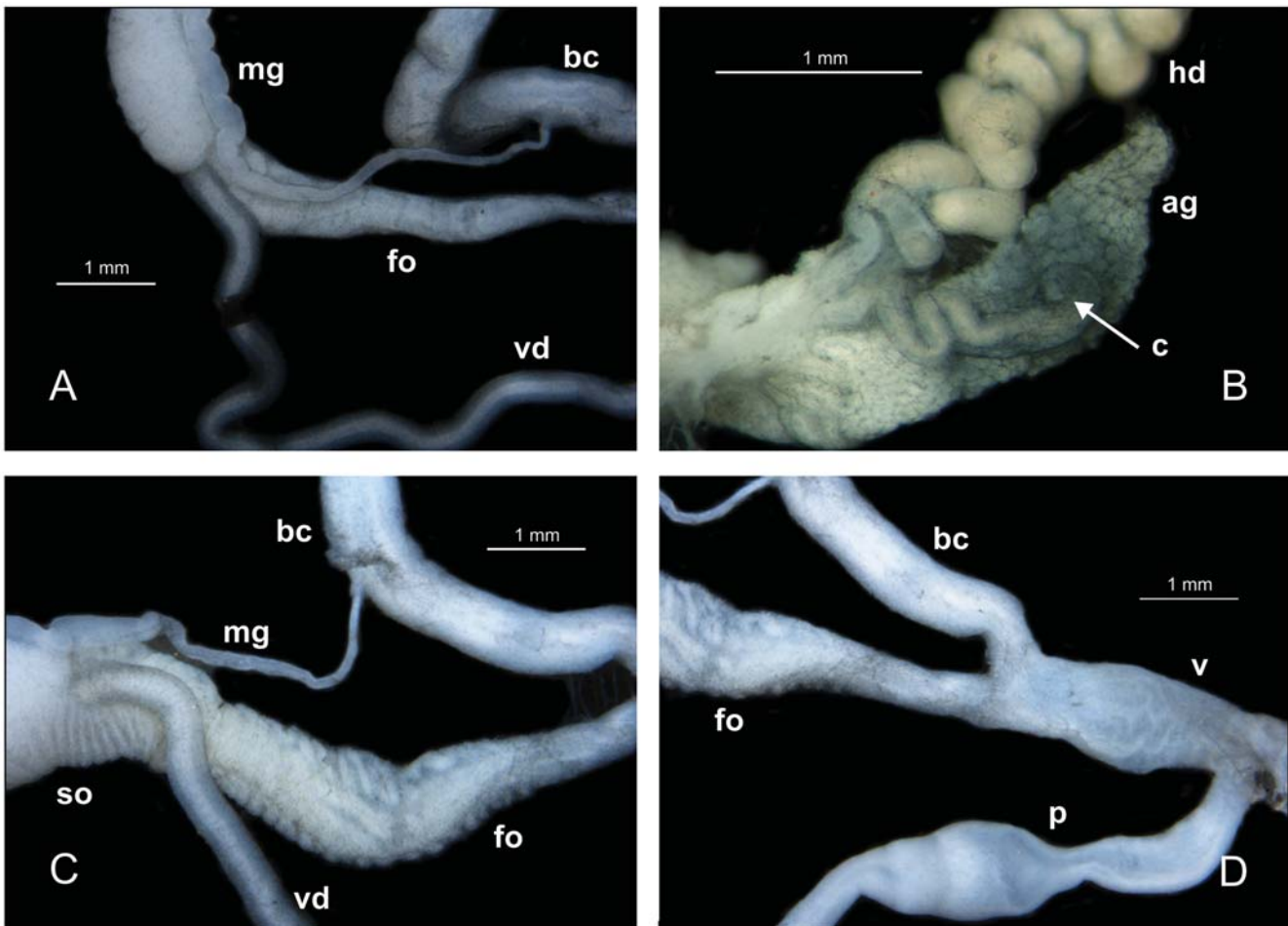
Figure 11. *Vestia gulo*. Reproductive system development, showing selected important parts. **A**, **B**. Stage A M3. **C**, **D**. Stage A M6. Abbreviations: ag, albumen gland; bc, bursa copulatrix; c, carrefour; fo, free oviduct; hd, hermaphroditic duct; p, penis; so, spermoviduct; v, vagina; vd, vas deferens. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

parts. Primordia of the clausilium, folds and lamellae appear during the first stage of formation of the ultimate whorl of the shell and their formation is very fast (Edlauer, 1941; Sulikowska-Drozd, 2009b). During formation of the closing apparatus and change of the shell outline into its final spindle-like shape, the animal is susceptible to damage of the delicate lamellae, which may result in deformations and ultimately death. The acceleration of growth of the ultimate whorl with its closing apparatus would be of a great advantage. The trade-off between growth and maturation is expressed differently in snails that grow and mature in parallel. Here, growth is considerably slowed during maturation (Maltz, 2003a, b and references cited therein).

Theoretical models predict that delayed reproduction and/or iteroparity should be adaptations to temporally uncertain environments (Tujapurkar, 1990; Wilbur & Rudolf, 2006). Koons *et al.* (2008) showed, however, that such uncertain environments could cause a delay mainly in semelparous organisms and in those iteroparous organisms whose juveniles are more likely to survive, compared to adults (e.g. as in some insects). Consequently, the uncertain environment could not lead to the evolution of delayed reproduction by iteroparous organisms whose adults are more likely to survive than juveniles, such as in the Clausiliidae. Thus, the delayed maturation of clausiliids cannot be explained by these models. We suspect that the delay in maturation decreases the time required to build the closing apparatus which reinforces the shell, thus

increasing the snail's chances of surviving and producing offspring in consecutive seasons.

Protandry and simultaneous hermaphroditism

According to Likharev (1962) the clausiliids, like most hermaphroditic pulmonates, are protandrous, since maturation of spermatozoa precedes that of ova. The statement is based on the fact that some time elapses between the exchange of spermatophores and egg production. In *Vestia gulo* and *V. turgida*, male and female reproductive organs develop at the same rate. The situation is similar in three other clausiliids (Maltz & Sulikowska-Drozd, 2008), as well as in *H. obvoluta* (Maltz, 2003a) and *H. lutescens* (Koralewska-Batura, 1994). In *Succinea putris* and *Oxyloma sarsi* (Jackiewicz & Zboralska, 1994), initially the rate is the same and later the male organs start developing faster. In *S. putris*, there is a strong preference for smaller individuals to play the active role during mating – they mount the shell of larger individuals and initiate copulation, even if reciprocal sperm exchange is the rule (Jordaens, Pinceel & Backeljau, 2005). Conchologically subadult *A. arbustorum* have been observed to copulate with adults (Baur, 1984); the copulation is reciprocal and after some time both partners lay eggs which would indicate that they have fully developed reproductive systems and are developmentally equal (although this has not been anatomically confirmed).

In *V. gulo* and *V. turgida*, male and female germ-line cells develop at the same rate. Only at the stage of spermatozoa is the differentiation of male cells (spermiogenesis) slightly earlier relative to the female line, because of the different mechanism of maturation of spermatocytes and oocytes. The reason for this difference is most probably due to the process of vitellogenesis, which is complicated and time- and energy-consuming. However, there are no distinct testis and ovary stages in the development of the gonad, contrary to the situation found in *Arion ater* (Lusis, 1961, after Tompa, 1984), where the oocyte stage follows the distinct stages of spermatogonia, spermatocytes, spermatids and spermatozoa. The clausiliid gonad combines the functions of testis and ovary from its earliest stages. Similar arrangements have been observed in *H. obvoluta* (Maltz, 2003a) and *V. pusilla* (Mazurkiewicz & Pokryszko, 2005). In this context, it is difficult to refer to protandry *sensu stricto* in the Clausiliidae and other hermaphroditic pulmonates showing the same pattern. The term 'slight protandry' proposed by Jordaens, Dillen & Backeljau (2007) seems more appropriate.

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