

Mating behaviour clarifies the taxonomy of slug species defined by genital anatomy: the *Deroceras rodnae* complex in the Sächsische Schweiz and elsewhere

JOHN M. C. HUTCHINSON¹ & HEIKE REISE²

Senckenberg Museum für Naturkunde Görlitz, PF 300 154, D-02806 Görlitz, Germany

¹ majmch@googlemail.com

² heike.reise@googlemail.com

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> Abstract

Collections of *Deroceras* from the uplands south of Dresden (along the Czech-German border) revealed two similar species differing in mating behaviour. The ranges interdigitate, but the species never occurred together. Species A has a wide sarcobelum held leftwards over its head and the everted penes are fully visible from above. It most resembles *Deroceras praecox*, occurring 100 km further east, whose anatomy and courtship behaviour are nevertheless consistently distinct; if these are different species, species A appears endemic to the Sächsische Schweiz. In species B, courtship and copulation take longer. Its sarcobelum is narrower, with a much enlarged base, and is directed forward or to the right. Most distinct is that the penes evert downwards and coil round each other for an additional revolution; their eversion is hidden from above except for the hand-like penial gland. This species is conspecific with Swiss, German and Austrian populations of *Deroceras rodnae*, but distinct from eastern populations, which more closely resemble *D. praecox* and species A. Unpublished molecular analyses support this division. Nevertheless, identifying non-mating animals by genital anatomy can be difficult: the base of the sarcobelum provides the best character. Western populations of *D. rodnae* should be termed *Deroceras juranum* Wüthrich, 1993.

> Kurzfassung

Paarungsverhalten klärt Taxonomie von genitalanatomisch definierten Nacktschnecken-Arten: der *Deroceras rodnae*-Komplex in der Sächsischen Schweiz und anderswo. – Aufsammlungen von *Deroceras* im Hügelland südlich von Dresden (entlang der deutsch-tschechischen Grenze) ergaben zwei ähnliche Arten, die sich im Paarungsverhalten unterscheiden. Ihre Areale greifen ineinander, gemeinsame Vorkommen wurden aber nicht entdeckt. Art A hat ein breites Sarcobelum, das beim Paarungsvorspiel nach links über den eigenen Kopf gelegt wird und die bei der Kopulation ausgestülpten Penes sind von oben voll sichtbar. Am ähnlichsten ist Art *Deroceras praecox*, die 100 km weiter östlich vorkommt, sich aber dennoch in Pisanatomie und Paarungsvorspiel unterscheidet. Falls Art A und *D. praecox* unterschiedliche Arten sind, könnte Art A endemisch für die Sächsische Schweiz sein. Bei Art B dauern Vorspiel und Kopulation länger. Das Sarcobelum ist schmäler, hat eine deutlich vergrößerte Basis und wird nach vorn oder rechts ausgestreckt. Der deutlichste Unterschied liegt aber in der Kopulation, bei der beide Penes nach unten ausgestülpt und um eine zusätzliche Windung verdreht werden. Mit Ausnahme der handähnlichen Penisdrüsen sind sie von oben nicht sichtbar. Diese Art ist identisch mit Populationen von *D. rodnae* in Deutschland, Österreich und der Schweiz, aber verschieden von östlichen Populationen, die mehr *D. praecox* und Art A ähneln. Nicht veröffentlichte molekularbiologische Untersuchungen unterstützen diese Teilung. Außer bei der Paarung kann die genitalanatomische Unterscheidung schwierig sein; die Reizkörper-Basis stellt das beste Merkmal dar. Westliche Populationen von *D. rodnae* sollten *Deroceras juranum* Wüthrich, 1993 genannt werden.

> Key words

Agriolimacidae, courtship, *Deroceras juranum*, *Deroceras praecox*, Elbsandsteingebirge, Erzgebirge, Děčínské stěny, etho-species, genitalia, sibling species.

Introduction

From the time of Linnaeus reproductive organs have been widely used to define and classify species. This might in part be merely because they are complex and well-defined structures but current thinking is that they also evolve more rapidly than many other morphological features as a result of some form of sexual selection (EBERHARD 1985; HOSKEN & STOCKLEY 2004; HUBER 2004). There is little support for the common belief that genital morphology evolves to prevent interspecific crosses (SHAPIRO & PORTER 1989) and it can be difficult to judge how much morphological divergence is sufficient to prevent successful mating. The species that we consider in this paper provide an example of how simple observations of mating behaviour can identify which genital characters determine incompatibility. In addition mating behaviour itself provides further characters to differentiate the species, although in this example these differences might not contribute to species recognition.

Slugs are typically harder to identify than snails because they lack the characters provided by a shell. The genus *Deroceras* is extreme in that the more than 100 species show very little variation in external appearance despite considerable, sometimes spectacular and bizarre, interspecific variation in their genitalia (WIKTOR 2000; REISE 2007). One problematic species pair is *Deroceras rodnae* Grossu & Lupu, 1965 and *Deroceras praecox* Wiktor, 1966. *D. praecox* has a curved pocket beyond the insertion of the penial glands at the end of its retracted penis (Fig. 6A, B) and is restricted to around the Sudeten Mountains, whereas *D. rodnae* lacks this pocket and is widely distributed from Romania to the Alps, with reports also from Spain. The two species do not occur sympatrically although we have recently identified narrow hybrid zones in the Babia Góra mountains of Poland and in the Mala Fatra mountains of Slovakia (REISE & HUTCHINSON unpublished). The suspicion might be that *D. praecox* is just a local variant of *D. rodnae* that happens to have a recognisable difference in its genitalia (REISE 2001).

The uncertainty has been partially resolved by observing their mating behaviour. These simultaneous hermaphrodites have a prolonged courtship phase of mating when the slugs lie close alongside each other in a yin-yang configuration and each partner protrudes from its genital pore a tongue-like sarcobelum with which it strokes the other, transferring a secretion (REISE 2007). This is followed by the copulation itself when the partners simultaneously evert the rest of their penes and sperm is rapidly transferred reciprocally from penis to penis. REISE (1995) noted that the species differ in the way the sarcobelum strokes the partner and

in the duration of courtship prior to copulation. The latter leads directly to mating incompatibility between *D. praecox* and *D. rodnae*, because, even if the faster species everts its penis, the partner is not yet ready to do so and thus cannot receive the sperm. However, REISE (1995) studied only non-adjacent populations of each species. Non-adjacent populations of *D. rodnae* also seem difficult to mate together (REISE unpublished), so it could be that there is much allopatric differentiation. Certainly there is further interpopulation morphological variation within what is conventionally considered *D. rodnae* (e.g. the population from the Mala Fatra mountains of Slovakia, named *Deroceras fatrense* Mácha, 1981, which has a larger sarcobelum).

This paper initially concerns *Deroceras* collected from an upland area called the Elbsandsteingebirge running along the German-Czech border west and east of the river Elbe (Fig. 1) and from the adjacent eastern end of the Erzgebirge (sites 12 and 15). The more familiar name Sächsische Schweiz describes the German side of the Elbsandsteingebirge and the Czech side is often referred to as the Děčínské stěny. *Deroceras rodnae* had already been reported from one site in this area to the east of the Elbe (site 3; REISE & SCHNIEBS 1997), we had since found another population nearby (site 4) and it was also known from an adjacent area to the east, the Lužické hory (sites 1 and 2; REISE 1995). Near the west of our area, *D. rodnae* had been recorded from the south-eastern edge of the Erzgebirge, near Teplice (I. FLASAR, personal communication). Recently we discovered other populations to the west of the Elbe that morphologically seemed allied to *D. rodnae* or *D. praecox*, but untypical (an unpublished MS of V. HERDAM refers to slugs at site 11 as *D. praecox*). This prompted us to examine their mating behaviour, which enabled us to distinguish two species, called here species A and species B.

In the Discussion we consider whether these deserve to be recognised as new species or merely as variants of *D. rodnae* or *D. praecox*. We will conclude that species B and many western populations hitherto considered *D. rodnae* should now be called *Deroceras juranum* Wüthrich, 1993, a species described on the spurious grounds of body colour from the Swiss Jura mountains (WÜTHRICH 1993) but later synonymised with *D. rodnae* (REISE 1997). Species A is closest to *D. praecox* and *D. rodnae* s. s. from the east: it is unresolved whether this population in the Sächsische Schweiz merits specific rank.

Tab. 1. Localities of species A and species B (= *D. juranum*) from the Sächsische Schweiz and environs (mapped in Fig. 1). Latitude and longitude are based on the projection WGS84. *N* is the number of individuals identified by dissection or from observing mating behaviour.

Site no.	Latitude, longitude	Collection date	Species	<i>N</i>	Habitat
1	50° 49' 54" N, 14° 29' 33" E	04.07.85–11.05.99	B	20	Meadow, conifer plantation, beech trees.
2	50° 50' 30" N, 14° 27' 26" E	17.05.94	B	5	By stream, mixed wood.
3	50° 56' 09" N, 14° 15' 31" E	04.05.96–19.11.96	B	21	By stream, deciduous wood.
4	50° 55' 29" N, 14° 15' 29" E	03.10.04	B	1	Mixed wood.
5	50° 54' 34" N, 13° 52' 10" E	03.06.06	A	2	By stream, edge of deciduous wood.
6	50° 52' 18" N, 13° 53' 57" E	04.06.06	A	4	By stream, meadow under deciduous trees.
7	50° 50' 40" N, 14° 00' 27" E	04.06.06	A	1	Roadside clearing in conifer plantation.
8	50° 44' 04" N, 13° 56' 57" E	20.04.07	B	11	By stream and meadow, edge of deciduous wood.
9	50° 46' 57" N, 13° 59' 53" E	20.04.07	B	6	Fen around spring, edge of deciduous wood.
10	50° 52' 44" N, 14° 12' 04" E	20.04.07	A	2	By small stream in conifer plantation.
11	50° 53' 57" N, 14° 10' 02" E	20.04.07	A	24	By streams, gorge, alongside mixed wood.
12	50° 48' 43" N, 13° 47' 13" E	25.04.07	B	1	By stream through field, deciduous trees.
13	50° 52' 57" N, 13° 58' 32" E	10.05.08	A	16	By stream and in road drainage ditch, in wood.
14	50° 51' 24" N, 14° 02' 27" E	10.05.08	B	8	By stream, edge of woodland.
15	50° 51' 52" N, 13° 48' 59" E	11.05.08	B	7	By stream, mixed wood.
16	50° 48' 39" N, 13° 54' 45" E	11.05.08	A	1	By stream, marsh by edge of conifer forest.
17	50° 50' 12" N, 14° 21' 43" E	17.10.08	B	3	Riverside vegetation, alder.
18	50° 52' 18" N, 13° 59' 38" E	16.05.09	A	9	Grassy track. Hedgerow. By stream in wood.
19	50° 51' 06" N, 13° 59' 41" E	16.05.09	A	2	By stream in mixed wood.
20	50° 50' 02" N, 13° 58' 54" E	16.05.09	A	3	By stream with scattered trees. Mowed grassland.
21	50° 49' 22" N, 13° 59' 18" E	16.05.09	A	20	Along stream with few trees. Deciduous wood.

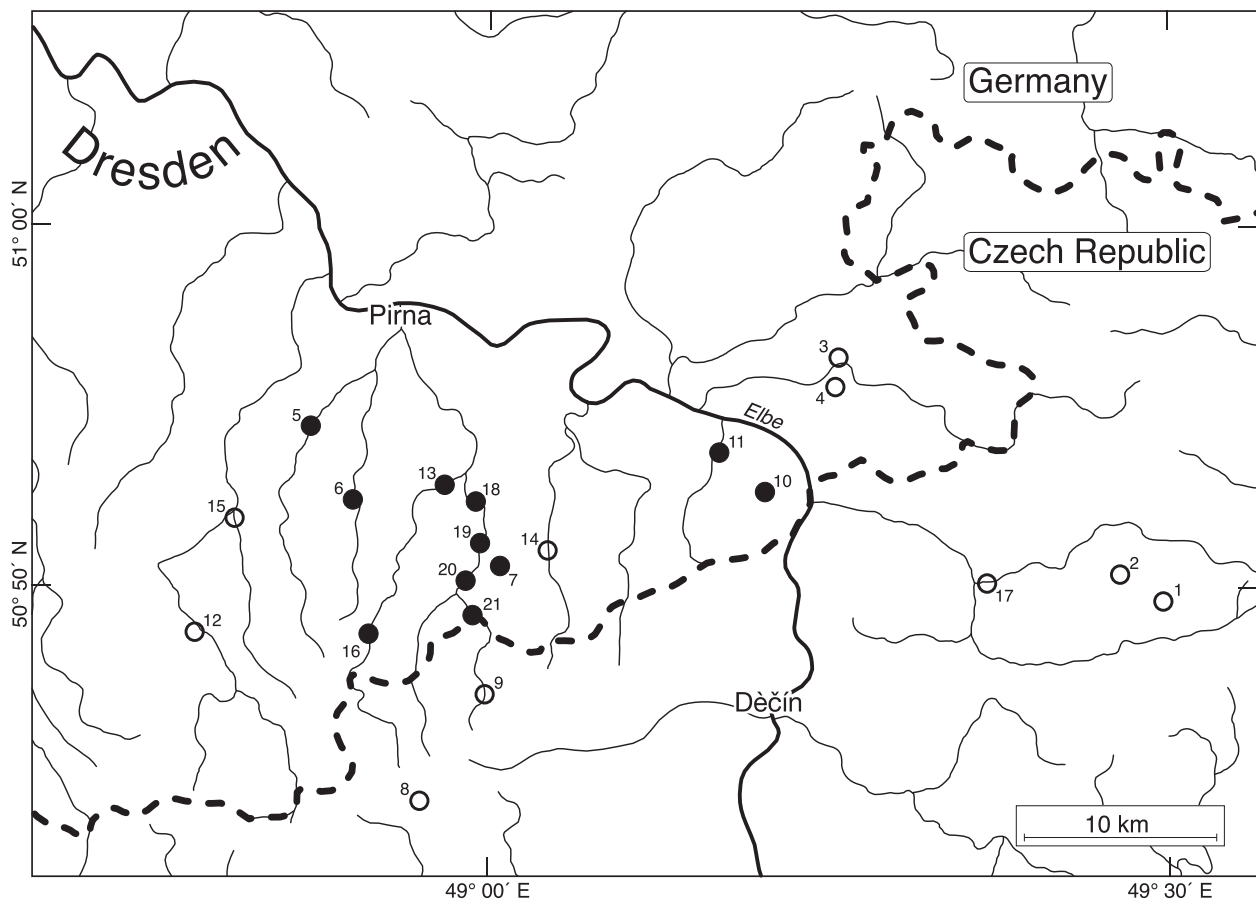


Fig. 1. Distribution of species A (●) and species B = *D. juranum* (○) in the Sächsische Schweiz and environs. The dashed line indicates the Czech-German border, and solid lines indicate streams and rivers. Table 1 provides further details of localities.

Methods

Field collections were targeted at *Deroceras* and concentrated on damp areas along streams with some broadleaved trees to provide suitable litter; our experience with *D. rodnae* elsewhere is that these slugs can be found most readily in such habitats and even in the middle of a dry day. Turning fallen leaves in the dampest hollows was the most successful tactic. However, in wet weather the species appeared in other habitats, sometimes in abundance, including at drier sites in mixed woodland, in a woodland clearing, along a hedgerow and on a grassy track. We avoided synanthropic habitats, where *Deroceras reticulatum* (O. F. Müller, 1774) is liable to occur. Searches were mostly in spring, when *D. rodnae* and its relatives are adult, but small specimens were sometimes reared in the laboratory for a few weeks. They were kept in Petri dishes furnished with damp tissue paper, a dead beech leaf, and small quantities of rolled oats, cat-food pellet, lettuce and carrot; dishes and contents were replaced twice a week.

We kept some slugs isolated in such Petri dishes and then staged matings by putting pairs of animals in clear plastic containers 113 × 113 × 35 mm, usually provided with damp tissue paper and a leaf. These were absent and the container was instead sprayed with water when we attempted video recordings of the underside of the mating pairs. We tried to maintain temperatures between 15° and 20 °C, and we kept the light low at least until mating was well under way, when it would be increased so as to make better video recordings. Typically we set up several pairs of slugs each day, regularly observing them and noting down precourtship behaviour. When a pair had formed a yin-yang configuration, the lid of the container was removed, and if necessary inverted, to allow video recording.

Prior to 2008, matings were recorded on an analogue Hi8 videocam (Sony CCD-VX1E) often using interval mode (2 s of recording every 30 s). In 2008 and 2009 the matings were studied more intensively using a digital video recorder card (ISIS TFS 406) allowing four-channel recording, up to 25 frames per s, and a resolution of 768 × 576 pixels. Input was from the analogue videocam or from video cameras (Fujitsu CG-311 and Sentech P63CT) with Fujinon YV10x5B-2 lenses.

From 2008 and 2009 we have video recordings of seven matings of species A (two of which included a view from below), eight of species B (three with a view from below), and two heterospecific courtships. Only two of these matings involved the same pair of animals, one individual of which was also involved in another mating with an individual previously involved

in a heterospecific courtship. From 2007 we have video recordings of nine matings of species A (but in only two was the copulation recorded in full), and six of species B. We also utilised video recordings made less systematically in earlier years of matings between specimens collected elsewhere.

Definitions of the mating phases follow REISE (2007): precourtship stops and courtship starts at the start of protrusion of the second sarcobelum, courtship ends and copulation starts with eversion of the rest of the penis, copulation ends when neither penis is in contact with the partner, "mating" refers to mating behaviour whether or not it is successful.

Slugs were mostly killed by immersion in carbonated water followed by the stepwise addition of ethanol up to a concentration of 70%. They were then dissected by cutting the body wall along the left margin of the sole and over the head (WIKTOR 2000). Genitalia were drawn using a camera lucida attachment to an Olympus SZX9 binocular microscope with ACH1X objective.

Results

Mating behaviour of species A

Precourtship in species A can be very short: one pair had protruded both sarcobela within two minutes of being placed together and both protrusions were always within half an hour of our first noting that an animal was following the other. Close trail following (one slug following immediately behind the other, with occasional contact from head to tail) does occur, but typically does not persist as much as in many other *Deroceras* species. Either the follower begins to overtake so that it slides along the body of its putative partner or the partner turns round to form a circle in which each partner inspects the other's tail. In the latter case, this leads to mutual inspection of the partner's right flank and then protrusion of the sarcobela within a minute or so of one another.

The everted "tongue" of the sarcobelum is axe-head shaped with the blade flattened and broadly expanded; individuals vary in whether the blade is a simple trapezoid shape or more flared as in Fig. 2A. It is distinctive of species A that the sarcobelum is held curved to the left over the animal's own head so that the blade wraps across the partner's back, mostly immediately posterior to the mantle, but sometimes over the tail. This requires the tongue of the sarcobelum to twist (Fig. 3B). In some individuals the application was persistently along the flank, but still posterior to the mantle. In one pair in which the sizes were mismatched

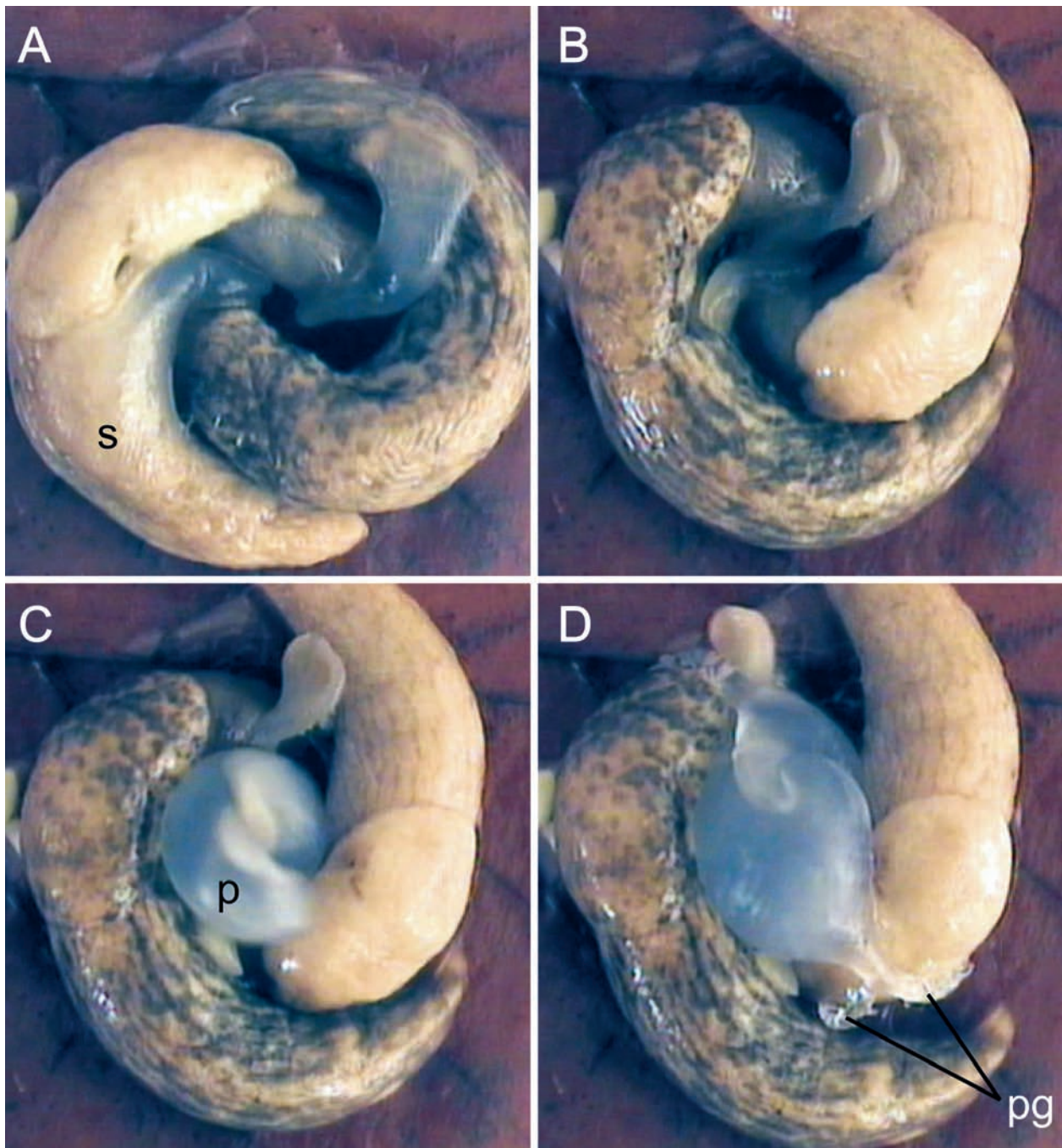


Fig. 2. Stills from a video recording of a mating of species A from site 13. (A): The middle of courtship with sarcobela fully extended (the tongue of the darker slug is marked 's'). (B): Immediately prior to copulation with the sarcobelum bases now opposed and the sarcobela tongues partially retracted. (C): The middle of penis eversion immediately before the penial glands start to evert; the penis marked 'p' belongs to the darker slug. (D): Full extent of penis eversion, with two branches of the penial gland of the darker slug marked 'pg'.

there seemed to be problems for the sarcobelum of the larger individual to bend sufficiently for the blade to make proper contact over the smaller individual, but nevertheless that mating was successful. Throughout courtship the sarcobelum pulsates with a regular beat (mean rate = once per 3.0 s), with the tongue lengthening and its blade dilating over the partner's back, alternating with a contraction drawing it back. Muscular ripples across the blade are also apparent.

It may be only after protrusion of the sarcobela that the animals assume a tight yin-yang configuration with each one's tail wrapped around the other and the heads pressed together (Fig. 2A). This "wheel" rotates steadily, but the rate tends to decrease; for instance, one pair took 68 s to make a full revolution in the second minute of courtship, but later this had increased to 375 s. One successfully mating pair was atypical in the rotation sometimes stopping and contact being

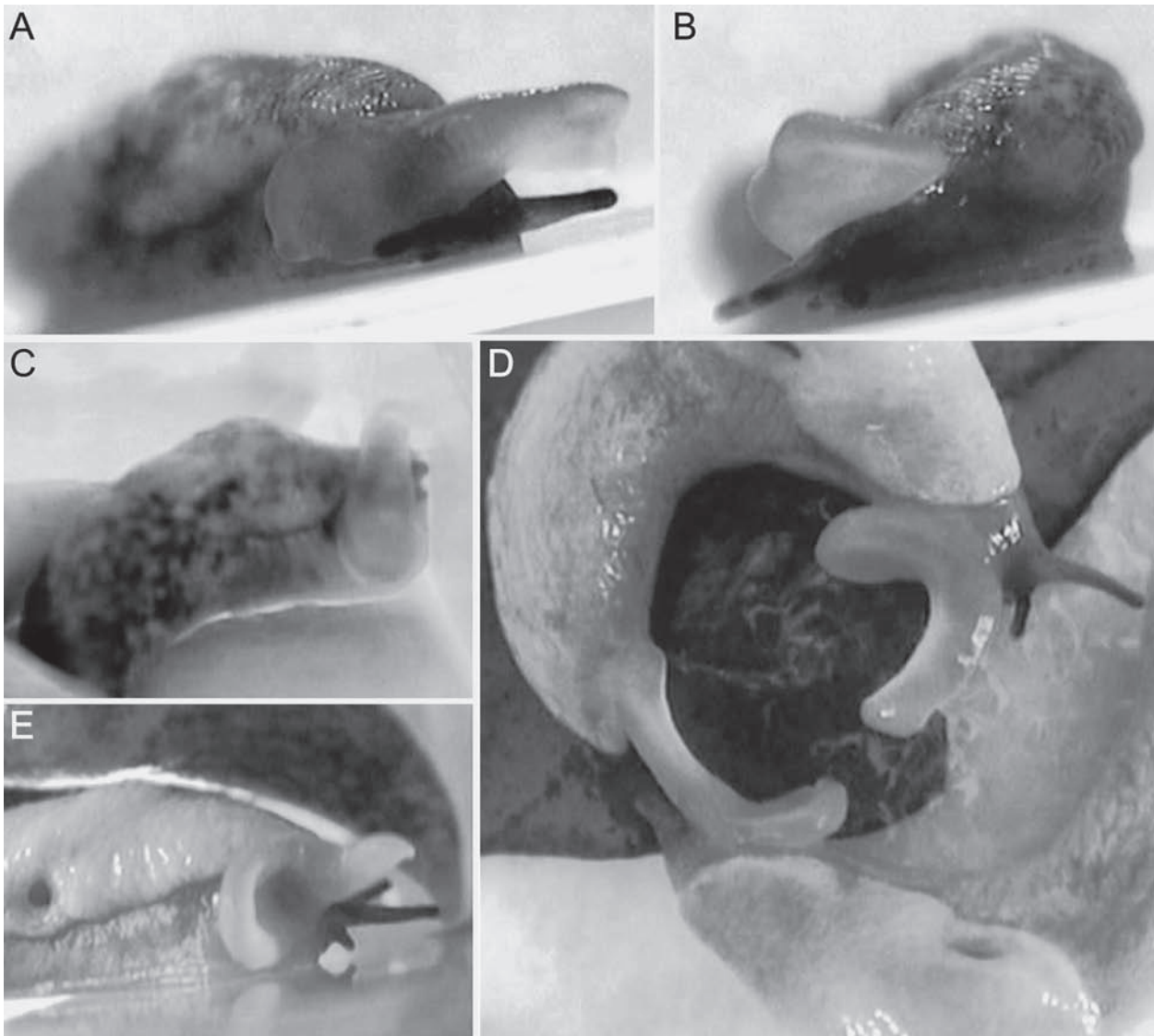


Fig. 3. Stills from video recordings of atypical matings that exposed the shape of the sarcobelum. (A–C): Species A from sites 18 (A, B) and site 16 (C). It is clearest in (C) that the base is a flat plate extending little beyond its site of attachment (a slight rim is visible in the dorsal view in Fig. 2A). (A) shows the teat-like protuberance sometimes present at the ventral corner of the sarcobelum base. (B) reveals the twist to the sarcobelum tongue. (D, E): species B (= *D. juranum*) from site 15, showing the larger ear-like structure, with a prominent thickened rim.

lost except via the sarcobela; perhaps it was because the partners were rather different in size, or because they were mating on a smooth vertical surface. During courtship, the tentacles and the lips of the mouth continually probe over the partner's flank.

The base of the sarcobelum is a flat round plate with a slight rim (Fig. 3C) that in dorsal view forms the appearance of a heel directed posteriorly (Fig. 2A). There may sometimes be a small teat-like protuberance at the ventral corner (Fig. 3A). In some matings the bases of the two sarcobela touch along their posterior edge almost throughout courtship, whereas in others each individual is positioned more posterior to the other (i.e. they overlap more), so that the sarcobela are out of contact (Fig. 2A). However, prior to copulation the partners slide back relative to the other

(i.e. decrease overlap) so that the posterior rims of the sarcobela do touch. And further backward movement leads to the base of the sarcobelum pressing against that of the partner side-to-side (Fig. 2B). Eversion of the rest of the penis always starts from such a position, but the position may be taken up once or twice without eversion occurring. At such times, a little of the rest of the penis may temporarily evert (visible only from underneath), as if reaching to touch that of the partner, and with some individuals this may also happen earlier in courtship when the bases of the sarcobela approach each other. Just before full eversion, the sarcobela contract somewhat. From protrusion of the second sarcobelum to eversion of the rest of the penis took 18–60 min in the 12 couples in which we timed both events (median = 32 min). One courting

pair failed to copulate and gave up after 165 min: they repeatedly brought the bases of the sarcobela together but full eversion never occurred.

Penis eversion at copulation is sudden, simultaneous and proceeds rapidly without pause. The main body of the penis extrudes backwards and upwards, and then curves outwards in an arc so that it hooks around the similarly curved penis of the partner (Fig. 2C). Only 0.5 s after the start of copulation, the long fingers of the penial gland evert and shoot over the anterior mantle of the partner (Fig. 2D). Also at this stage a pocket everts, forming a hook at the end of the penis which weakly interlocks with that of the partner (Fig. 2D), or one may cover the other. Penial eversion is complete 0.9–1.2 s after its start. Without pause, the main bag of the penis starts to contract while the glands are still everted and the penes are dragged apart from one another by movement of the head. From the start of penial eversion until loss of contact of penis with the partner takes 9–20 s and it is then another 15–65 s until the penes retract out of sight. Slugs may then spend some minutes apparently eating the mucus deposited during the courtship, and they may also double up and lick their backs, perhaps to remove material deposited by the partner's sarcobelum.

In one pair the two penes failed to engage each other as they everted. Despite slipping past each other just before eversion of the penial gland and terminal hook, both these structures everted as normal. Presumably sperm was not exchanged.

Mating behaviour of species B

All components of the mating of species B are more prolonged than that of species A. This includes the precourtship (i.e. before sarcobelum protrusions). Typically the first indication of precourtship is that one slug follows behind the other, and then they circle around each other at some distance, but not in any regular pattern; initially it seems merely a tendency to return to the site where mucus has already been deposited. Eventually they synchronise their positions sufficiently that each investigates the tail or right flank of the other, then perhaps inspect the other's genital pore, and then they form a yin-yang configuration with the tentacles touching the other's flank beneath the pneumostome. However, the start is hard to define and at any of these stages it is not unusual for the process to break off and later restart from the beginning: consequently from first interest to yin-yang formation is of very variable duration (from 9 min to an hour or two). This yin-yang configuration will start to revolve but it may be some time (median = 11 min) before a sarcobelum protrudes.

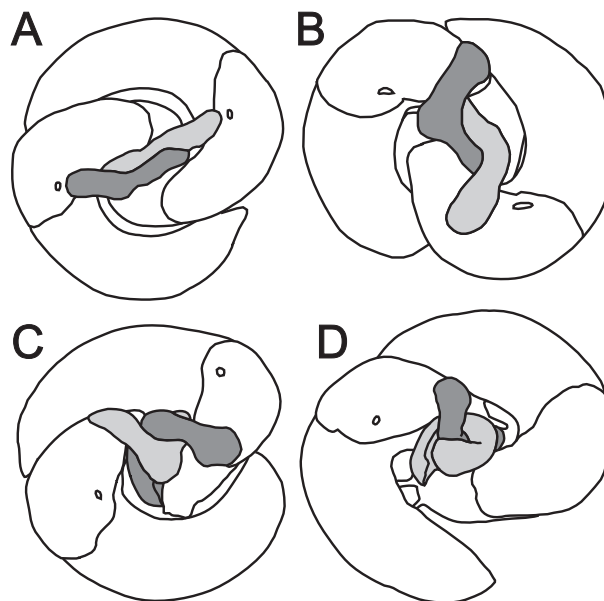


Fig. 4. The diversity of positioning of the sarcobelum during courtship in species B (= *D. juranum*); the sarcobelum of each partner is differently shaded for clarity. (A): Site 14. (B): Site 15. (C) and (D) are from one mating from site 8, respectively in early courtship and later when one sarcobelum tongue has slid onto the partner's sarcobelum. Traced from video stills.

The sarcobelum of this species consists of a long thin tongue directed anteriorly and a broad ear-like base that extends posteriorly from the genital pore (Fig. 3D, E). The tongue does not assume its full length when first protruded and initially it rubs the partner's right flank while the "ear" covers the partner's genital pore. The second sarcobelum typically emerges a minute or two after the first (range = 29 s to 10 min). The "ears" press tightly together and may remain so till copulation, so the animals overlap less than in species A. After both have protruded, the sarcobelum tongues initially continue to stroke the right flank of the partner, but within a few minutes usually are redirected dorsally to rub around the pneumostome. Or, in some matings, one or both tongues are bent sharply to the right and rub the dorsal surface of the mantle anterior to the pneumostome. In part where the tongue is applied may depend on the relative sizes of the partners and whether the recipient remains upright or leans over on its left side: the partners are often asymmetric in these respects. However, there is also considerable intersite variation: at site 14 the tongues were most often held straight forward, but sometimes bent sharply right, whereas at site 15 the converse applied (Fig. 4A, B). At sites 8 and 9 they were also held perpendicular to the right, but there the two tongues lay alongside one another (Fig. 4C); they would be applied to the anterior margin of the mantle, but later in courtship the tips of one or both tongues could curve further back and slip

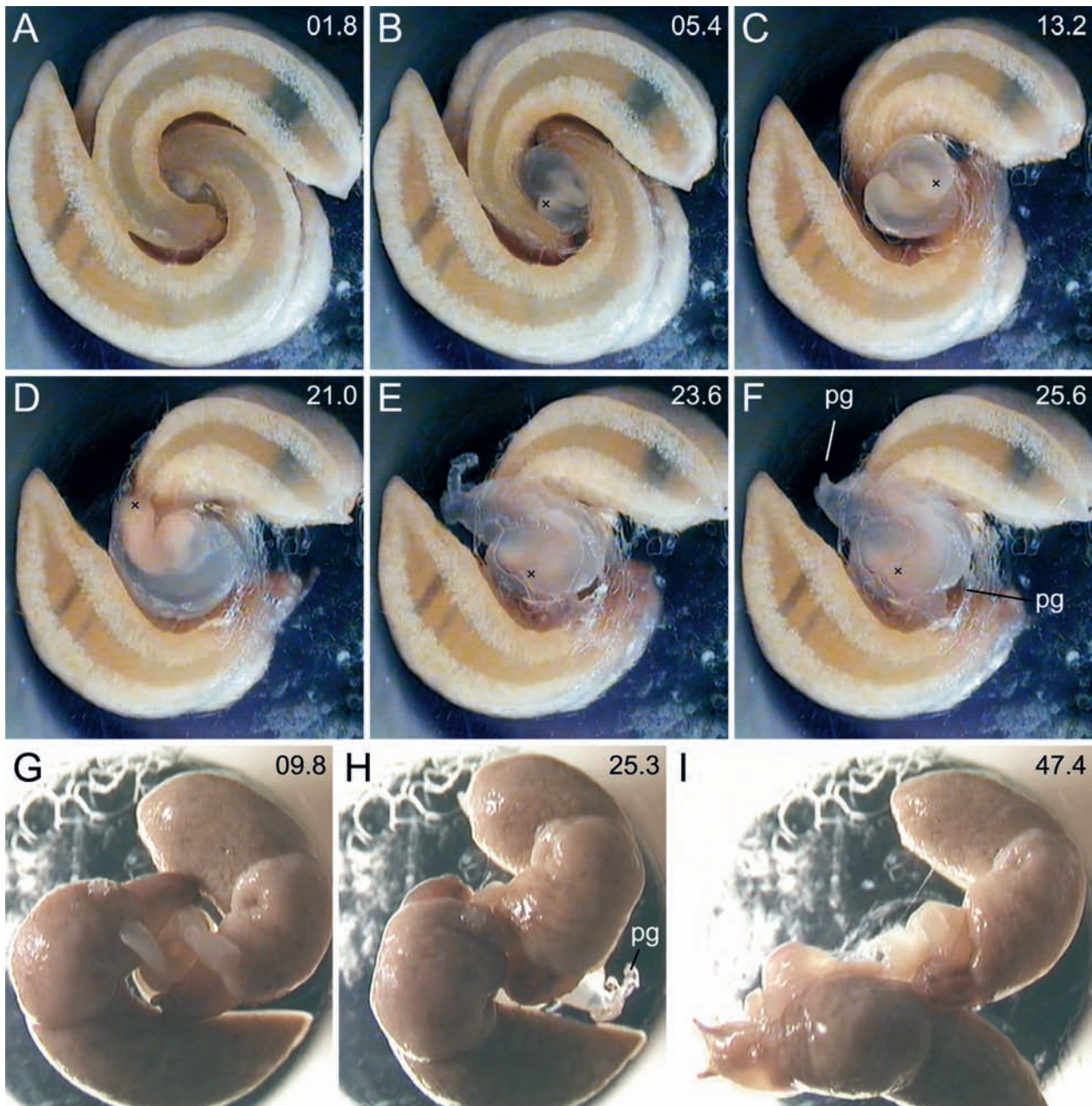


Fig. 5. Stills from a video recording of a copulation of species B (= *D. juranum*) from site 15, from below (A–F) and above (G–I). Figures in the top right of each frame give seconds from start of penis eversion. (A) The slug at the top has just started to evert its penis; just to the right of this can be seen the pore through which its partner will shortly evert its penis. (B) Both penes are everting; in frames (B–F), the penis of the slug at the bottom is marked with a cross. (C) This position is held for some seconds. Note that the heads have each retracted backwards since the start of eversion. (D) After the restart of eversion, the marked penis has everted to the point where the penial glands will evert. (E) These penial glands have started to evert and the tip of the penis has already expanded by another half turn, its maximal extent. (F) The penial glands of both partners are now everted, but those of the upper slug are harder to spot as they lie under the partner's sole. (G) The little that may be apparent of the copulation from above: the heads have retracted back (earlier they fitted snugly in the curve of the partner's body) and the dorsal parts of the sarcobela have pulled slightly apart (in some pairs it was much further apart), with a part of the everted penis visible lying under the tentacle of the right-hand slug. (H) One penial gland appearing like an upturned hand appearing from under the partner: the sarcobela are now partially contracted. Shortly the slugs will turn their heads to the side to vigorously apply their mouths to the everted penes. (I) The slug at the bottom has just pulled its head away to separate the entwined penes.

onto the partner's sarcobelum (Fig. 4D). Thus at the end of some courtships the sarcobela were wrapped around each other. At sites 14 and 15, there was no such change in configuration throughout the long courtship except that in a few matings the bases did

briefly separate early on as if the animals sought to reposition themselves; in one case this separation led to the partners losing the yin-yang configuration, so that the sarcobela were withdrawn before reengaging some minutes later.

The rubbing motion of the tongue of the sarcobelum is forward and back along its length, rather than a lateral stroke (similar to species A but with less dilation at the tip). The pulse rate appears not to vary systematically through courtship (mean rate = once every 4.6 s). The animals continue to circle throughout courtship, initially fast (e.g. one revolution in 101 s), but then slowing down (e.g. one revolution in 7 min: rates were much slower for pairs courting on the side of the container and one such pair stopped for half an hour until disturbed). The other movement, most clearly visible from below, is the tentacles continually reaching out and touching the right flank of the partner. Near the time of copulation, the lips around the mouth become protruded and touch the flank; they may also turn to the genitalia. Courtship continues for a median of 118 min (range 54–255 min).

Viewed from above, little of the copulation is apparent because, almost uniquely for *Deroceras*, the penes evert beneath the bodies. Usually eversion is roughly synchronised between partners, but in one mating the penis of one partner unilaterally everted a little four times within the four minutes preceding a normal synchronised eversion. Each penis everts in a circular arc, outwards, forwards and then back inwards, hooking round that of the partner (Fig. 5A–C), and the process is much slower than in species A. Within about 5 s, while the eversion continues below, from above all one can see is that the heads of each animal make an anti-clockwise movement (i.e. a retraction) and the ear-like sarcobelum bases separate along their dorsal margin. In some matings these pivot back so far that the bases lie almost side-upwards, consequently appearing so prominent that casual observation might mistake them for the rest of the penis (as in Fig. 8M). In contrast with species A, there is not always much reduction in the size of the protruded sarcobela tongues until later, when the penial glands evert (Fig. 5G). After the penes have everted one revolution (c. 9 s after eversion started), there is a distinct pause of about 6 s (Fig. 5C), when the penes tighten somewhat against each other (i.e. a slight contraction without retraction). One pair repeatedly attained this state but then retracted fully, so it may be a decision point.

More than another full revolution of penial eversion then occurs, more rapidly than earlier. The penes form a double helix, wider ventrally and tapering up to the point of insertion. After about half this revolution, 4 s after the end of the pause, the penial glands start to evert (Fig. 5D–F). Often they evert beyond the partner and the fingers curve up and inwards towards the partner's head, but usually not touching it. The fingers of the everted gland are short, so the distinctive appearance from above is like a hand directed palm upwards reaching round from under the partner's head (Fig. 5H); that is almost all that can be seen of the

everted penis from that normal direction of viewing. In *Deroceras panormitanum* (Lesson & Pollonera, 1882) we know that the glands transfer a secretion over the partner as they evert (BENKE et al. in press), so the lack of contact in species B is curious. However, often only one upturned hand or none is observed, and in the two copulations observed also from below, the explanation was that the glands everted under the sole of the partner (Fig. 5F), which could be the intended site of application. The glands take about 3 s to evert fully, by which time the rest of the penis is also maximally extended. The animals have by then started to move their open mouths vigorously up and down over the base of the partner's everted penis.

Some contraction of the penes is apparent after about 4 s of full eversion, but they remain twisted together and their separation only begins a few seconds later when one partner moves its head sharply away. Often more than one tug is necessary to break apart the double helix, so that from initial eversion to separation lasted a median of 40 s (range 36–64 s). Penis retraction into the body can take another half minute or longer. After copulation at least one slug is usually avid in eating up the mucus left at the site of copulation, and they may also bend round to lick their own flanks or genital pores.

Heterospecific crosses

We set up two heterospecific matings, both of which proceeded into courtship but no further. As in its homospecific matings, species A protruded its sarcobelum rapidly after contacting its partner's flank. However, early on there was some difficulty in remaining in contact with the species B partner, which appeared still to be in a circling phase of behaviour (although periodically inspecting the partner's tail and later flank). Since species B was at this stage often extended straight, in order to make contact species A had to direct the tongue of its sarcobelum forward rather than over its head as in its homospecific matings. It took some time for the second sarcobelum to be everted (> 41 min) and in this period contact was occasionally lost and then species A sometimes retracted its sarcobelum briefly. Once started, species B took some minutes to protrude its sarcobelum fully. Eventually both pairs did manage to achieve a yin-yang position with the sarcobelum of species A directed over its head and that of species B forward to the right, but both touching either side of the posterior edge of the partner's mantle. However, periodic rearrangements in the position of the sarcobelum bases suggested some conflict or confusion: mostly the bases covered each other (cf. species B matings) but also sometimes they lay edge to edge

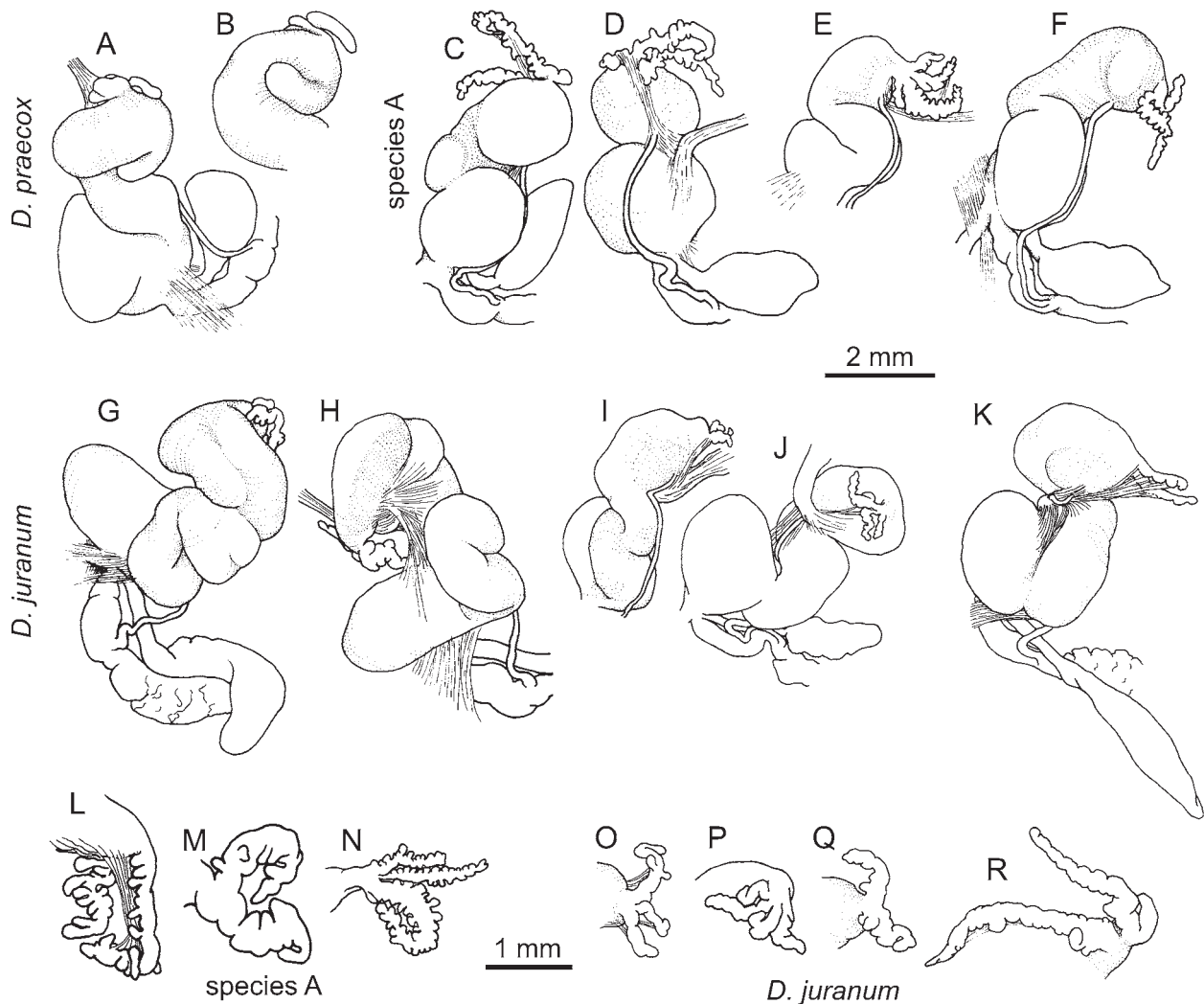


Fig. 6. Distal genitalia, with close-ups of penial glands, of *D. praecox* (A, B), species A (C–F, L–N) and species B = *D. juranum* (G–K, O–R). (A, B): Two views of a *D. praecox* penis from Ryzmburk, 9 km W of Náchod, Czech Republic, collected by BRABENEČ, showing the typical coiled end. (C, D): Two views of a species A penis from site 11: typically short and with two long branches of the penial gland. (E): Another short penis, but untypical, hand-like penial gland, from site 6. (F): A longer species A penis, again from site 11. (G, H): Two views of one typically long *D. juranum* penis, from site 8. (I, J): Two views of another long penis, from site 9. (K): A shorter *D. juranum* penis, again from site 8. (L–N): Three penial glands of species A from site 13 (retractor muscles shown only in (L)); (N) is untypical in having more than two branches. (O–Q): Three penial glands of *D. juranum* from site 8 (retractor muscles shown only in (O); (P) is same individual as (G) and (H)). Note that (Q) has only two branches (cf. species A), but they are short. (R): An untypical specimen of *D. juranum* with two long branches of penial gland, from Altenburg near Moosach, ESE of Munich, Bavaria.

(cf. species A matings). One arrangement observed in both pairs involved the posterior edge of species A's sarcobelum base pushing back the ear-like flap of species B, so that the latter was bent double over itself.

In one pair it was species B that appeared to break off the courtship (91 min since it protruded its sarcobelum, and 132 min since its species A partner protruded), whereas in the other pair it was species A (98 min since it protruded and 54 min since its species B partner protruded). In comparison, only two out of 30 homospecific matings failed to proceed to copulation (not all successful) after the start of courtship. The one mating between slugs of different populations of

species B (sites 14 and 15, 16 km apart on different river systems) was successful and normal.

Genital anatomy

Our observations of the mating behaviour have also revealed much about the anatomy of the genitalia as they serve their function and in how the species differ in this respect. However, it is infeasible to get slugs to evert their genitalia for routine species identifications, so we sought consistent morphological differences in the retracted genitalia. Figures 6 and 7 show the genitalia of each species. What follows here is based solely

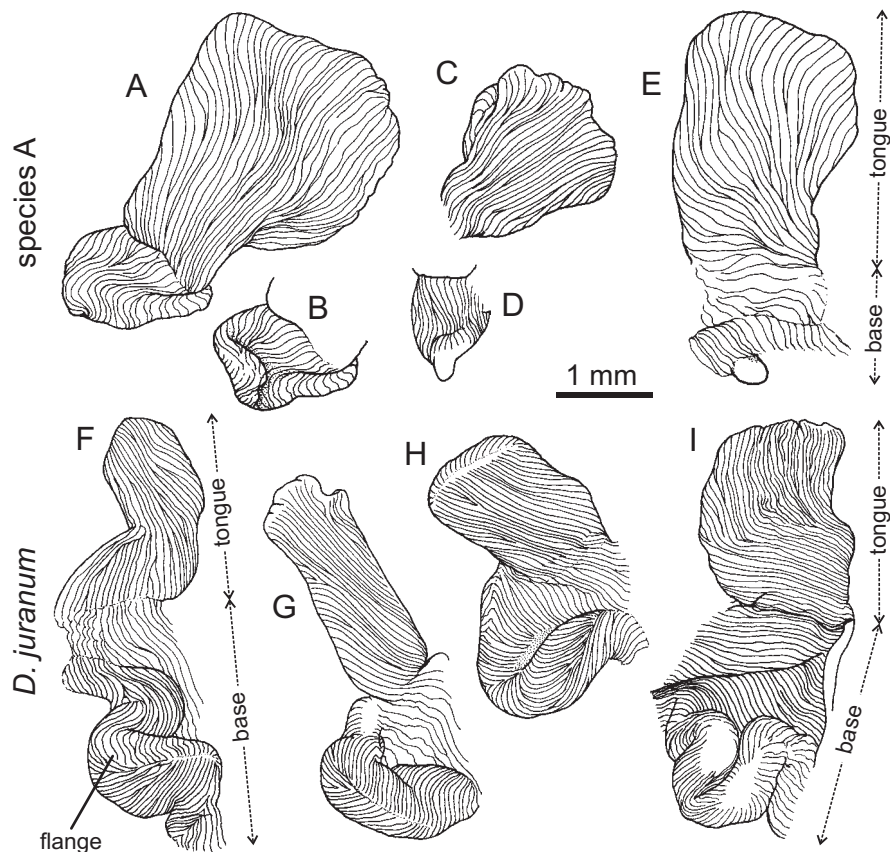


Fig. 7. Sarcobela of species A (A–E) and species B = *D. juranum* (F–I). (A): Sarcobelum with broad fan-like tongue typical of species A, from site 11. (B): The same view of the sarcobelum base of (A) but showing the small thickened edge reflected forwards. (C): Another fan-like sarcobelum tongue, from site 12, but with a teat-like protuberance on base (D). (E): Also from site 12, a less flared sarcobelum tongue, again with a protuberance on base. (F): Sarcobelum of *D. juranum* penis in Figs 6G and 6H (site 8) showing typical long parallel-sided tongue and large thickened flange to base. (G): Another typical example, from site 9. (H): An untypically short sarcobelum tongue from site 14. (I): The sarcobelum from the same Bavarian specimen as Fig. 6R: representative of this population, the tongue is broad, short and also thinner, thus reminiscent of species A.

on material from the Sächsische Schweiz and we consider in the Discussion the extent to which these characters are useful elsewhere.

One of the most fundamental interspecific differences is that the penial glands evert after half a revolution of penial eversion in species A, but one and a half revolutions in species B, so we expected the retracted penis of species B to be longer. Most specimens of species B indeed were safely recognisable by having a long convoluted penis (Fig. 6G–J), but others, even from the same site, had as short a retracted penis as species A (Fig. 6K). However, even the longest penes of species A (e.g. Fig. 6F) are never as long as those of typical species B. The two copulations of species B observed from below also suggested that it may have a longer continuation of the penis beyond the insertion of the penial gland. However, when the penis is retracted the penial glands insert at its tip in both species. Further dissection of this part of the penis revealed various internal pockets and folds that might generate the hook-shaped tip of the everted penis, but

their configuration was confusing and inconsistent even within a population.

HUDEC (1970), following WIKTOR (1966), gives the direction of coiling of this part of the retracted penis as a character to distinguish *D. rodnae* and *D. praecox*, but we could not recognise any such difference in our pair of species. In both species the penis everts in an anticlockwise direction when viewed from above; since in species A the penis everts upwards and in species B downwards, it forms respectively a right-handed and a left-handed helix. Unfortunately when the penis is retracted it folds up in a zigzag way that is hard to classify in terms of handedness.

The larger sarcobelum base in species B was another prominent interspecific difference apparent in the matings (Fig. 2). When retracted, sarcobelum base and tongue can be seen by cutting open the swollen part of the penis near the genital opening. In species B, the prominent hind margin of the base visible during courtship forms in the retracted state a thick lip-like flange (Fig. 7F–I). This flange may (Fig. 7H) or may

not (e.g. Fig. 7F) connect to the tongue and it continues, diminishing in size, into the thinner tubular part of the penis. In species A (Fig. 7A–E), this structure is much smaller, does not continue into the thinner part of the penis, and is often drawn into a small teat-like protuberance (Fig. 7D, E) that presumably corresponds with the ventral protuberance sometimes seen when the sarcobelum is protruded (Fig. 3A). Even in the retracted state it is apparent that the tongue of the retracted sarcobelum is fan shaped and wide in species A, but longer, narrower, and roughly parallel sided in species B.

The penial glands (Fig. 6) provide a slightly more accessible distinguishing character. Species A typically has two long fingers, whereas species B has two to four shorter fingers. However, there is overlap in length and species A can also have up to four fingers (Fig. 6N), so only some specimens can be determined with confidence by this character.

WIKTOR (1973) mentions the penial retractor muscles as distinguishing *D. praecox* and *D. rodnae*, but we could find no consistent difference in our species. Another taxonomic character in *Deroceras* is the caecum of the gut; in both our species it was a very small pocket or merely a bulge.

Discussion

Are our species *D. praecox* or *D. rodnae*?

The type locality of *D. rodnae* is in Romania, but we lack material from so far east. However, we have video recorded full matings from populations just east of the range of *D. praecox* (N of Lúčky-kúpele, Chočské mountains of Slovakia and several sites in the mountain range Babia Góra in Poland), and from a population further south in Slovakia (near Banská Hodruša, Stiavnické pohorie), and also courtships

from a population further east in Poland (near Krzemienna, Pogórze Dynówskie). At copulation the penes are visible from above, fitting the pattern of species A, as does the duration of courtship (20–60 min: REISE 1995, VISSER & REISE unpublished). In preserved material from these sites, from several sites along the southern border of Poland east of the range of *D. praecox* (see WIKTOR 1973) and also from the Bükk mountains (Hungary) the morphology of the sarcobelum base is similar to species A, although unsurprisingly the *D. rodnae* populations span a somewhat wider range of variation. The configuration of their penial glands is usually not the two long fingers typical of species A, although it does show variation, even within a population. Similarly, although we have not observed populations of *D. rodnae* which apply the sarcobelum tongue over the owner's head like in species A, the shape of the sarcobelum tongue, and how it is applied, also show considerable variation between these populations (Fig. 8). Morphologically *D. rodnae* is a species defined almost by the absence of distinctive morphological characters. Therefore it may be justifiable to view species A as a local subspecies of *D. rodnae* with a distinctive courtship and minor differences in genital morphology; the same status might be given to *D. fatrense*, for instance, or even *D. praecox*.

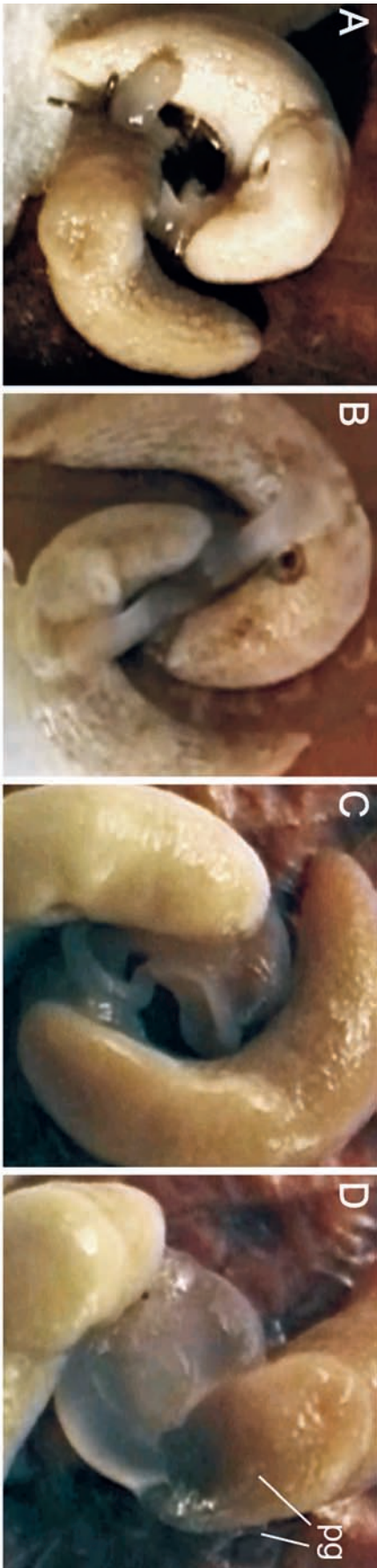
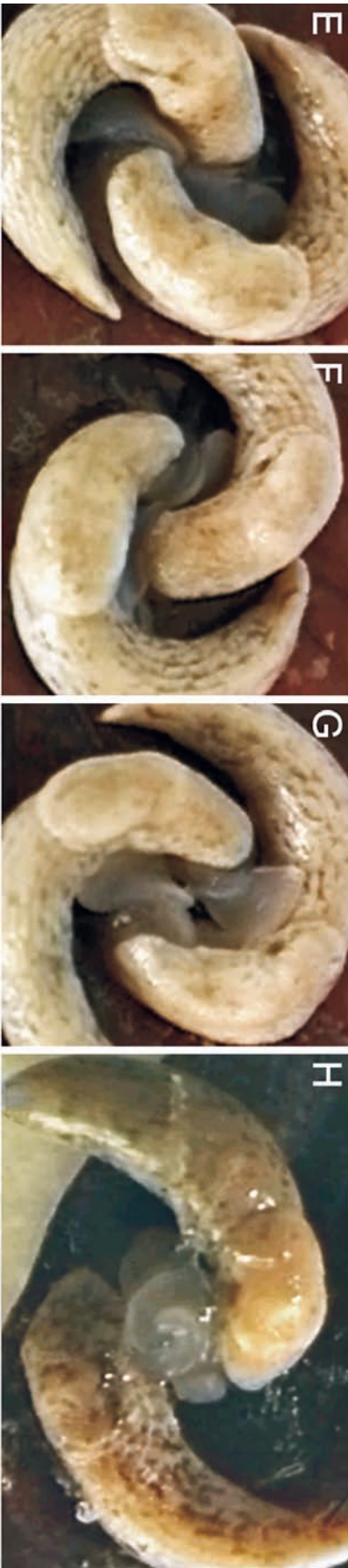
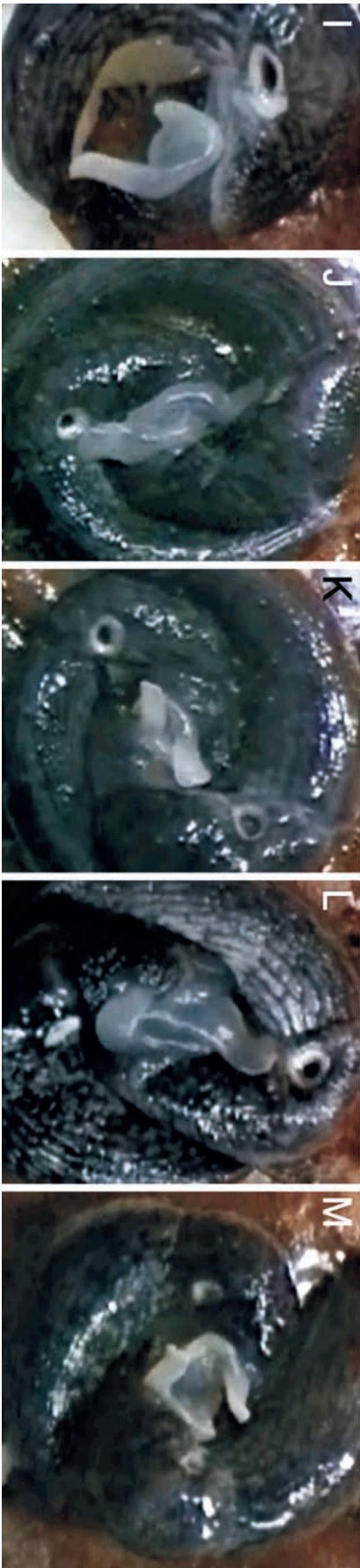
The nearest (and westernmost) locality of *Deroceras praecox* that we can personally confirm is only 100 km to the east of the Elbe, at the foot of the Sněžka in the Krkonoše Mountains (HUDEC 1967; mapped in REISE et al. 2005). HUDEC (1967) also gives a record of *D. praecox* genitalia from the Czech-German border at the Elbe (i.e. probably opposite site 10: Fig. 1), but it is suspicious that the penial glands in his illustration are so short and maybe a swelling could have been over-interpreted as the distinctive coiled end to a *D. praecox* penis. HUDEC's only *Deroceras* specimen from this locality in the National Museum of Natural History Prague matches also in collection date but is a previously undissected *Deroceras turcicum* (Simroth, 1894) (REISE unpublished), so this anomalous record perhaps needs confirmation. Like the eastern popula-

Fig. 8. Video stills of mating in *D. rodnae* s. s. (A–D), *D. praecox* (E–H) and *D. juranum* (I–M). (A–C): Sarcobela of *D. rodnae* from three distant populations: (A) short conical tongue (normally the tongues lie against each other, hiding the thumb-like base visible here) from near Krzemienna, Pogórze Dynówskie, Poland; (B) long narrow tongue from N of Lúčky-kúpele, Chočské mountains, Slovakia (near the range of *D. fatrense*); (C) cup-shaped tongue applied to the flank, from beside stream Zakulawka, SE flank of Babia Góra, Poland. (D): Copulation in same mating as (C), with tips of two branches of penial gland (pg) indicated. (E–G): Stages of a single courtship of *D. praecox* from SW of Mionší, Moravskoslezské Beskydy, Czech Republic, showing positioning of sarcobelum tongue over the owner's head (E), shovel-like application under the partner typical of most of courtship (F), and application to the flanks shortly before copulation (G). (H): Copulation of *D. praecox* from Horný Vadičov, 14 km NE of Žilina, Slovakia. (I): Sarcobelum of *D. juranum* from Combe Grède (type locality) (cf. Fig. 3D, E). (J–L): Diversity of courtship configurations in couples from Swiss Jura: (J) most usual pattern (from Hasenmatt, NW of Solothurn); (K) same mating as (J), perhaps during an attempt at copulation; (L) one tongue gripping base of partner's sarcobelum (from Combe Grède); a pair from Twannberg, SW of Biel, showed a pattern as in Fig. 4B. (M): Start of copulation in same mating as (L), when sarcobelum bases pivot apart.

D. juranum

D. praecox

D. rodnae s. s.



tions of *D. rodnae* considered above, *D. praecox* resembles species A in that the courtship is short (REISE 1995) and the copulation is rapid with the penes visible from above (Fig. 8H). More particular resemblances with species A are that the sarcobelum tongue is often held over the owner's head during courtship (Fig. 8E), and it is large and broadening towards the tip; also the sarcobelum base, when protruded, is small with a heel-like rim when viewed from above (cf. Figs 8G and 2A), and in its retracted state it also resembles species A. The configuration of the penial glands is often the two long fingers typical of species A. Thus species A seems to be more closely related to *D. praecox* than to any populations of *D. rodnae* that we have examined. However, *D. praecox* has a distinctive curved pocket at the end of the retracted penis (lacking in species A) and the sarcobelum tongue in *D. praecox* is applied under the sole of the partner until shortly before copulation, when it touches only the flank, never the back of the partner as in species A (Fig. 2A). Because in both these respects *D. praecox* is very consistent across its range (REISE unpublished), the definition of *D. praecox* would have to be significantly broadened to include species A. We refrain from judging whether to call species A either *D. praecox*, or a variety of *D. rodnae*, or a new species. Data that might resolve the issue could come from molecular evidence, an ability of *D. praecox* and species A to interbreed in captivity, or the discovery of populations between the Sächsische Schweiz and the Krkonoše Mountains that show characters intermediate between species A and *D. praecox*.

We also have video recordings of matings of *D. rodnae* from west of the range of *D. praecox* (from the Jura mountains of Switzerland – see REISE 1997 for details of most sites – and from the north side of the Sondersberg, in the Chiemgau Alps of Bavaria). Although all recordings are from above, it is clear that these populations copulate like species B in that the penes are scarcely visible (except for the sarcobela). We also observed the backward motion of the heads, the pivoting apart of the sarcobelum bases (Fig. 8M), hand-like penial glands appearing from underneath, and the application of the mouth to the entwined penes. Also the duration of courtship agrees (REISE 1997). In two slugs from the Jura in which the base of the sarcobelum became fully visible during courtship, it is just as large as in the Sächsische Schweiz, but it differs in forming a hook along its ventral margin (Fig. 8I, cf. Fig. 3D, E). Such minor differences seem unsurprising in populations 600 km apart. Similarly it should not be surprising if conspecific animals from distant populations show some difficulties in mating, but species B and western *D. rodnae* from elsewhere are able to proceed better than do species A and B with each other. Thus in a mating of species B from site 1 with a

slug from the Jura mountains, the copulation appeared successful even though the penes spiralled round each other not around a vertical axis as normal, but around one pointing more horizontally. Two matings of species B from site 3 with a slug from Goldersbachtal, near Tübingen, Swabia (the same animals in each case) also both lead to a copulation, although the penes appeared not to engage properly so that they pulled apart, probably before sperm exchange.

We have examined preserved material not only from the western populations mentioned above but also from Suhl (Thuringia), Meiningen (Thuringia), Weissenfels (Thuringia, SW of Leipzig), Gera (Thuringia; VON KNORRE 1969), Lörrach (Württemberg, NE of Basel), Munich, Schärding (Austria, S of Passau), and Horn (NW of Vienna; JORDAENS et al. 1998); it all appears conspecific. In the slugs from Horn and from both Bavarian populations the sarcobelum tongue is shorter, much wider and also thinner (e.g. Fig. 7I), thus reminiscent of species A. However, the sarcobelum base is consistently pronounced as in species B and distinct from species A or the eastern populations. (A specimen from the Spanish population at Capsacostas Pass reported as *D. rodnae* by CASTILLEJO et al. (1993), and matching the genitalia illustrated by them, is clearly not conspecific. Also seemingly distinct are another sample from northern Spain and a sample from near Milan that were labelled as *D. rodnae* in the collection of the Wrocław Museum of Natural History.)

We conclude that western populations of *D. rodnae* (e.g. from Switzerland and Germany) are conspecific with species B and are clearly not conspecific with *D. rodnae* east of the range of *D. praecox*. The morphological differences between western and eastern populations are mostly not absolutely consistent or may seem minor compared with the considerable interpopulation variation at smaller geographical scales. But what is critical is that the eastern populations show a copulation like that of species A, which is fundamentally different from that of species B and other western populations. A consequence is that crossing between western and eastern populations seems physically impossible. These populations also differ in the duration of courtship, another isolating mechanism. We henceforth refer to the eastern populations as *D. rodnae* s. s. The valid name of western *D. rodnae* s. l. becomes *Deroceras juranum* Wüthrich, 1993. This taxon was described on the basis of its dark coloration from a population in the Jura mountains of Switzerland (WÜTHRICH 1993). Although REISE (1997) subsequently showed the coloration to be a Mendelian inherited colour morph, the name has formal taxonomic priority. Video recordings of *D. juranum* from the type locality itself confirm that the mating is like species B (Fig. 8M).

The splitting off of *D. juranum* from *D. rodnae* s. s. was also supported by an allozyme analysis in which the eastern populations of *D. rodnae* s. l. clustered with *D. praecox* and not with western populations (REISE et al. 2001). Sequences of two mitochondrial genes (VISSER, REISE & BACKELJAU unpublished) also imply this separation. A wider phylogenetic analysis using molecular methods should now examine whether there are other species to which *D. juranum* is more closely related than *D. rodnae* s. s. and *D. praecox*. Given the very different copulations, and the lack of distinctive morphological characters in *D. rodnae* s. s., it is conceivable that they are not so closely related.

REISE (1995) thought to have resolved whether *D. praecox* and *D. rodnae* were separate species when finding consistent differences in their mating behaviour. However, the populations of *D. rodnae* s. l. that she used for the behavioural comparison were all *D. juranum*. So it now turns out that there remains an issue of whether *D. praecox* and *D. rodnae* s. s. are specifically distinct. Our own research (VISSER, REISE & HUTCHINSON unpublished) indicates that adjacent populations of each species will copulate with each other, but the genital morphology of slugs collected from the wild suggests that the hybrid zone is remarkably narrow.

Broader lessons for taxonomy of *Deroceras*

Although the molecular data had made us suspect a fundamental split between western and eastern populations of *D. rodnae* s. l. for some time, we had until now not recognised any morphological differentiation. The breakthrough came from studying close-up video recordings of mating. Besides providing extra behavioural characters, these observations revealed the morphology of the genitalia when in use rather than packed away (and possibly further distorted to varying extents by the killing process). Furthermore, the observations facilitated interpreting the biological significance of morphological differences, and suggested which characters in preserved specimens might and might not be reliable in distinguishing species. Video was superior over direct observation in (1) allowing comparison of populations collected from far apart in different years, (2) enabling rechecking of characters not initially suspected to be significant, (3) slowing down the rapid copulation so that the details could be followed, and (4) facilitating observation from beneath a copulating pair. These improvements probably explain the discrepancies between the observations in this paper and REISE's (1995) direct observations of the copulations in *D. juranum* and *D. praecox*.

The copulations of our two focal species showed such fundamental differences that it was clear that

they could not mate successfully with each other: in particular, copulation in species B involved an extra revolution of penial eversion and the penis coiled downwards instead of upwards. However, we were not able to find a morphological character of the retracted genitalia that reliably indicated these particular differences. Although one might suppose that the shorter penial gland typical of *D. juranum* is an adaptation to pushing under the partner rather than spreading over it, the only other species of *Deroceras* that is known to use its gland in this way has particularly long glands (REISE et al. 2007).

Observations of the mating also revealed interspecific differences in the shape of sarcobelum base and tongue and these did translate into recognisable differences when retracted. Although in some populations outside our study area the sarcobelum tongue was not always a clear character distinguishing *D. juranum*, its sarcobelum base was consistently distinct. However, it is questionable whether differences in sarcobelum shape play much role in species recognition or incompatibility. Certainly there seems little selectivity about starting courtship: on four occasions slugs of species A elicited sarcobelum eversion even in individuals of *Deroceras reticulatum* (or possibly *Deroceras lothari* Giusti, 1971: their identification is problematic), which have a very different sarcobelum (long, pointed, conical, without a base). Species A and *D. juranum* continued to court together for long periods and the failure to initiate copulation seems as likely to be based on different timings or on chemical differences in the secretions as on any morphological incompatibility. REISE (1995) did sometimes observe that *D. praecox* everted its penis when courting *D. juranum* (earlier in courtship than the latter would normally copulate). A role for sarcobelum morphology in species recognition also seems incompatible with the variation in the way that *D. juranum*'s sarcobelum tongue is applied to the partner (Figs 4 and 8). Although this variation is predominantly between sites, there is an appreciable echo of it within sites. Furthermore, the interspecific matings implied that an individual could considerably adjust how it applied its sarcobelum. Further east, we have discovered sharp transitions between *D. rodnae* s. s. and *D. praecox* in the morphology of the sarcobelum and how they apply it, but mixed couples seem not to have particular problems with each slug courting in its own way: such matings can proceed to simultaneous penis eversion (VISSER, REISE & HUTCHINSON unpublished).

The reason behind the variation in how *D. juranum* applies its sarcobelum is unclear. Beyond the Sächsische Schweiz, also the shape of its sarcobelum varies considerably. Because it is the sarcobelum that is involved in courtship, some of the intrapopulation variation might be interpretable as condition-dependent

sexually-selected characters, but evidence is lacking of such condition dependence or even of mate choice. In any case this would not explain the interpopulation variation. There is even greater interpopulation variation within *D. rodnae* s. s. in sarcobelum shape and how it is applied (Fig. 8A–C). The variation could indicate that it matters little on what site the sarcobelum applies a secretion (cf. GOULSON 1993). But the courtship seems such an elaborate and costly behaviour that we also speculate whether each individual is attempting to manipulate the partner against the partner's best interests (e.g. to donate a surplus of sperm that the recipient can digest, or to utilise the received sperm for fertilisation). The intrapopulation variation might then reflect frequency-dependent benefits of using an unusual "fighting" style and interpopulation diversification might result from different outcomes of independent coevolutionary arms races fuelled by sexual conflict (REISE 2007; BENKE et al. in press, cf. KOENE & SCHULENBURG 2005; BEESE et al. 2006). Intraspecific interpopulation variation in genital morphology is being increasingly documented in other molluscan (e.g. LACE 1992; MADEC & GUILLER 1994; BAMINGER & HAASE 2000; FIORENTINO et al. 2008) and non-molluscan taxa (e.g. KAWANO 2004; HOLWELL 2008; SONG & WENZEL 2008), and probably is still underestimated (HUBER 2004), but the reasons behind the variation are not well established. Less has been documented about interpopulation variation in mating behaviour, except for acoustic signals, which are easier to record and quantify than other aspects of behaviour (VERRELL 1999).

There is a precedent for mating behaviour providing the key to slug taxonomy, albeit in a different family. Simple observations of the mating of the very common slug *Arion hortensis* Férussac, 1819 detected consistent differences in timing and genital morphology which implied that an additional two species had been previously unrecognised (DAVIES 1977). In that case the most useful morphological character for distinguishing the species turned out to be the part of the genitalia involved in attaching the transferred spermatophore. This character could play a role in species recognition. Arthropods provide other examples of "ethospecies", species that differ in their mating behaviour but are very similar in morphology (e.g. DEN HOLLANDER & DIJKSTRA 1974; UETZ & DENTERLEIN 1979).

Distribution and other open questions

Figure 1 shows that the distribution of the two species in the Sächsische Schweiz is fairly interdigitated. Although each river system tends to have a single species, the head waters of the Bahra stream provide one

exception (cf. sites 9 and 21). Nor have we detected any habitat differences between sites where we collected each species. Nevertheless no sites had both species, suggesting competitive exclusion; this is certainly worth further investigation, particularly along the Bahra. *Deroceras reticulatum* (or possibly *D. lothari*) co-occurred at two sites (12, 15) with *D. juranum* and at one site (21) with species A.

Deroceras juranum is known to be widely distributed elsewhere, but species A, if it is distinct from *D. rodnae* s. s. and *D. praecox*, could be endemic to the Sächsische Schweiz and to Germany, although site 21 is only 200 m from the Czech border. If species A is instead considered *D. praecox* or *D. rodnae* s. s., this would be the only known occurrence of either species in Germany. Conservation considerations would therefore argue strongly for further sampling to establish more precisely the distribution and habitat requirements. We had little success searching in the habitat of dissected sandstone plateau and dry conifer woodland for which the Sächsische Schweiz is best known and which is most intensively protected; almost all finds were in the richer valley bottoms and in the more rolling agricultural landscape south of Pirna. It would also be worthwhile to sample more widely, especially in the Czech Republic. Are there populations bridging the 100 km between the ranges of *D. praecox* and species A? Or, are there populations connecting species A and populations of *D. rodnae* s. s. through the more southerly parts of the Czech Republic?

The samples so far examined suggest that *D. praecox* and *D. rodnae* s. s. occupy the Carpathian and Sudeten mountains, whereas *D. juranum* occupies the Alps, extending northwards into southern Germany (as far north almost as Leipzig). Our study area is as far east as *D. juranum* is known to extend in the north of its range but to the south its occurrence near Vienna (JORDAENS et al. 1998) is further east. The ranges should therefore probably meet in the Czech Republic, and perhaps also Slovakia, Hungary or Austria, but the border or area of overlap needs much work to be delineated. One surprise was that the genital anatomy of a population from the Plješevica mountains of Croatia (i.e. S of the Austrian populations and connected by mountains to the Alps) appears to fit *D. rodnae* s. s.

Another area of uncertainty is the life cycle of each species in the Sächsische Schweiz. We know that in spring there are both adults and juveniles that soon mature in captivity. An October sample of *D. juranum* from site 17 included few individuals that were mature enough even to identify. So we suspect an annual life cycle but maybe some animals breed in autumn, and we do not know what proportion of the populations overwinter as adults or juveniles. WIKTOR (1973) claimed that *D. praecox* was mature in spring and *D. rodnae* mainly in autumn.

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