

# IS *LIMAX CINEREONIGER* WOLF, 1803, PRESENT IN ITALY? AN ANATOMICAL APPROACH TO THE STUDY OF ITALIAN *LIMAX* (GASTROPODA: PULMONATA: LIMACIDAE)

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*Abstract* An anatomical approach to the study of the slugs belonging to the genus *Limax*, based on the structures present in the inner walls of the penis, is proposed. These characteristics clearly allow the separation of the different species and they agree with the results obtained in genetic investigations. In this first contribution many populations of *L. cinereoniger* are reported from the north-eastern area of Italy (Veneto, Trentino-Alto Adige and Friuli-Venezia Giulia) and compared with other European populations. The diagnostic characters of *L. cinereoniger*, such as the sole colour (blackish lateral bands), the comparatively elongate penis (42–159mm), first crest much longer than the second one and the low and very short second crest and pleat are discussed and compared with those of similar species.

*Key words* *Limax cinereoniger*, genital anatomy, Europe, Italy.

## INTRODUCTION

Amongst the largest European invertebrates, one of the least-known groups is represented by the slug genus *Limax* Linnaeus, 1758. Indeed, in the past, the taxonomy of this group has been very confused and mainly based on variable and inconsistent external chromatic characters. In land snails and slugs, genitalia has usually been of great taxonomic significance, but in the genus *Limax* superficial examination of genital characteristics has not offered significant discriminating characters. Recent genetic and ethological investigations (Nitz *et al.*, 2010; Rowson *et al.*, 2014a) have provided new elements to better understand this group, but a deep comparative anatomical examination is still missing.

The first monograph of the Italian species belonging to the genus *Limax* was proposed by Lessona & Pollonera (1882), which recognised as valid 12 different species and numerous sub-specific taxa. A small number of other contributions, all very confused, were published in the following years (Colosi, 1920; Gambetta, 1932). The list by Alzona (1971) is largely based on the authors of the late nineteenth century. Only

at the end of the twentieth century did studies highlight the importance of some anatomical details, allowing the differentiation of some species and the description of new taxa (Giusti, 1968; Giusti & Mazzini, 1971; Giusti, 1973a, 1973b, 1976; Giusti *et al.*, 1985). The checklist by Manganelli *et al.* (1995), was also based on many unpublished data and lists 18 different *Limax* species. Recently, some authors have added other species to the Italian fauna (Reitano *et al.*, 2007; Falkner, 2008; Falkner & Niederhöfer, 2008; Nitz *et al.*, 2009; Heim *et al.*, 2010; Nitz *et al.*, 2010; Brandstetter, 2011). Finally, additional new taxa have been identified through genetic studies (Nitz *et al.*, 2010; Rowson *et al.*, 2014a), but not yet formally described. Overall, about 30 different species have been listed for the Italian territory (Bank & Neubert, 2017), however some need to be revised and a comprehensive review is lacking. In any case, the main problem is to establish the identity of many nominal species described in the past, that lack typical or topotypical specimens or their detailed anatomical data.

One of the problems that has never been adequately tackled is the true presence of *Limax cinereoniger* Wolf, 1803, in Italy. This species,

described on the basis of its external appearance (Wolf, 1803), has been defined anatomically by the greater penis length, if compared to that of *Limax maximus* Linnaeus, 1758 (Quick, 1960; Lupu, 1973; Kerney *et al.*, 1983; Wiktor, 1983; Gittenberger *et al.*, 1984; Falkner, 1990; Kerney & Cameron, 1999; Welter-Schultes, 2012). In Italy, the name "*cinereoniger*" has been repeatedly used, both in the past and recently, for specimens with the typical colour pattern, but without anatomical confirmations (Lessona & Pollonera, 1882; Marcuzzi *et al.*, 1970; Alzona, 1971; Cossignani & Cossignani, 1995; Decet & Fossa, 2001; Welter-Schultes, 2012; Brandstetter, 2015). Some authors, in absence of careful anatomical verifications, considered its presence in Italy as unconfirmed (Manganelli *et al.*, 1995; Bank & Neubert, 2017). Specimens similar to *L. cinereoniger* in their external colour and penis length, have been assigned to other entities, especially to *Limax bielzi* Siebert, 1873, although with doubt (Boato *et al.*, 1989; Dalfreddo *et al.*, 2000; De Mattia, 2003) and without verification of typical specimens. Indeed, genitalia of the Italian specimens seemed to be more like those of *L. bielzi* compared to those described for *L. cinereoniger*.

In the present study specimens of *L. cinereoniger* collected in northern and central European countries, certainly attributable to this taxon, are compared with others collected in Italy and in Balkan regions. This has allowed verification of the diagnostic characters of the genitalia of this taxon and confirms the presence of *L. cinereoniger* in Italy, although it is limited to the northeast.

## MATERIALS AND METHODS

Specimens were collected by hand from the ground, on trunks of trees and from under stones or rotten wood, in Italy and other European countries. Live photographs were taken using a film or digital camera.

Specimens used for anatomical examination were drowned or narcotised with ethanol, fixed in 75–80% ethanol and studied using a stereomicroscope. The body was dissected with very thin watchmaking tweezers. The animal and the anatomical details were drawn using a camera lucida.

Radula was obtained by dissecting out the buccal bulb, washing in distilled water, and then

mounted on copper blocks, spatter-coated with graphite, then with gold, and photographed using a scanning electron microscope.

The list of the material examined (see Appendix) includes only adult specimens (sp) checked anatomically (53 slugs from 47 sites), with well-developed penis and ovispermiduct (at least the prostatic portion of the ovispermiduct); no specimens with immature genitalia or unchecked anatomy are listed. Specimens with immature or unchecked genitalia are instead presented in the NE Italy distribution map (Fig. 8 B) with different symbols.

Terminology of genitalia (proximal and distal are related to the origin of the genital system): penis starts in its proximal portion, at the insertion of the vas deferens, close to the penial retractor muscle, and ends in its distal portion, close to the genital atrium (Figs 2 A, 10 A). For the internal structures of the penis (Figs 2 F, 10 A), the word "crest" is used for the high plica extended longitudinally from the proximal penis to the genital atrium, which is subdivided into a "first crest" and a "second crest", usually contiguous but separated by a contact point where the vertices of the two structures are not aligned with each other. The structure beginning near the second crest, running parallel to it but lower in height, is called "pleat" (in literature crest and pleat are usually not distinct and called "fold").

The material examined, preserved in ethanol, is placed in the following university, museums and private collections: C. Dalfreddo, Via Bagnols sur Ceze 21, Feltre, Belluno (CDC); F. Giusti, Department of Physical Sciences, Earth and Environment, University of Siena, Via P.A. Mattioli 4, Siena (FGC); G. Nardi, Via Boschette 8/A, Gussago, Brescia (GNC); I. Niero, Via Cici 17/1, Spinea, Venetia (INC); Friulian Museum of Natural History, Via Marangoni 39/41, Udine (MFSN); Museum of Natural History, University of Florence, Zoological Section "La Specola", Via Romana 17, Florence (MZUF; "GC" for number of collection of the specimens and "stub" for number of the radula mounted on copper block).

Abbreviations in the Figs and Table: bc: bursa copulatrix; c: callus; dbc: duct of the bursa copulatrix; fc: first crest; fo: free oviduct; osd: ovispermiduct; pa: papilla; pe: penis; pl: pleat; prm: penial retractor muscle; sc: second crest; vd: vas deferens.

## SYSTEMATICS

*Limax cinereoniger* Wolf, 1803

*Limax cinereoniger* Wolf, 1803: Die Würmer. In: Sturm T., Deutschlands Fauna in Abbildungen nach der Natur mit Beschreibungen, 6 (1): 7–8, 1 pl.

*Type locality* "Ober Krumbach bei Herfpruck" [Oberkrumbach near Hersbruck], Germany (Wolf, 1803); Germany (Quick, 1960; Wiktor, 1973, 1983, 1989, 1996).

*Type material* Unknown (Wiktor, 1973); probably does not exist (Wiktor, 1996).

*Differential diagnosis* Animal large, usually black or blackish in colour, with clearly visible keel (carina), attaining about 1/2 of the distance between back edge of the mantle and tip of the tail; sole usually with dark lateral parts. Long penis (42–159mm in preserved specimens), cylindrical, approximately as long as the body length; inner wall of penis with high and very long first crest, followed by a second, very short and low crest, found only in the distal tract close to the genital atrium; pleat low and poorly or not formed, present only in the last very short tract of the distal penis, running parallel to the second crest.

*Body* (Fig. 1) Large (living specimens up to 20cm), blackish or greyish in colour (exceptionally cream or reddish, Fig. 1 F, J–L), with uniform colour or with lighter sides or lighter spots on the body, rarely with lighter spots on the mantle or with black spots and/or dark bands on the body; prominent keel, usually whitish in colour (rarely blackish, exceptionally reddish), length about 1/2 of the distance between back edge of the mantle and tip of the tail. Cephalic tentacles greyish, labial tentacles paler. Sole divided into three parts by two longitudinal grooves; lateral parts dark grey or blackish, central part cream or whitish (sole entirely whitish in young specimens).

*Shell* Internal, fingernail shaped and nucleus placed near its posterior left margin, thickened and calcareous in the centre, thin and horny on its margins.

*Reproductive system* (Figs 2–6; Table 1) Hermaphroditic gonad (ovotestis) at (or near)

the apex of visceral sac; slender and convoluted hermaphrodite duct; large albumen gland, followed by a long ovispermiduct, consisting of a uterine and prostatic portions, followed at its end by a short free oviduct, ending in the genital atrium side by side the bursa copulatrix duct and penis. Large and sac-like bursa copulatrix, with rather short duct. Vas deferens shorter than the penis (ratio penis/vas deferens: 1.8–4.3), inserted asymmetrically in the proximal penis tip. Penis retractor muscle large, ending at proximal penis tip, close to the vas deferens. Long penis (42–159mm in preserved specimens), about as long as the body length, cylindrical, outside twisted and coiled, pimpled in some tracts. Internal proximal penis with high and very long first crest, followed by a second, very short low crest (ratio first/second crest: from 5.3 to 24.7), present only in the last short tract of distal penis. The contact point between the first and the second crest is low or quite low; the first crest ends right where the second one begins. The pleat is low and poorly evident (sometimes apparently absent), and it appears only in the last very short tract of distal penis walls, parallel to the second crest, close to the genital atrium. Very small papillae, more evident in the proximal portion, cover the internal surface of the penis, while prominent spaced transversal grooves interrupt the internal surface.

*Radula* (Fig. 7) More than 100 rows of teeth, each composed of about 140–170 teeth, with a single central tooth, many lateral teeth and many marginal teeth. Central tooth slightly smaller than laterals, tricuspid, with a long basal plate, a large and long mesocone followed, on each side, by two very small ectocones. Lateral teeth tricuspid, with large basal plate, a strong mesocone and a very small endocone and ectocone. Marginal teeth with reduced basal plate and a very long tooth, a long mesocone, pointed at tip, a small endocone and one (or sometimes two) very small ectocones. The transition from laterals to marginal teeth is gradual, with endocone disappearing at about the 30<sup>th</sup> lateral. Shape and dimensions of teeth are rather variable in various portions of the radula and in different specimens.

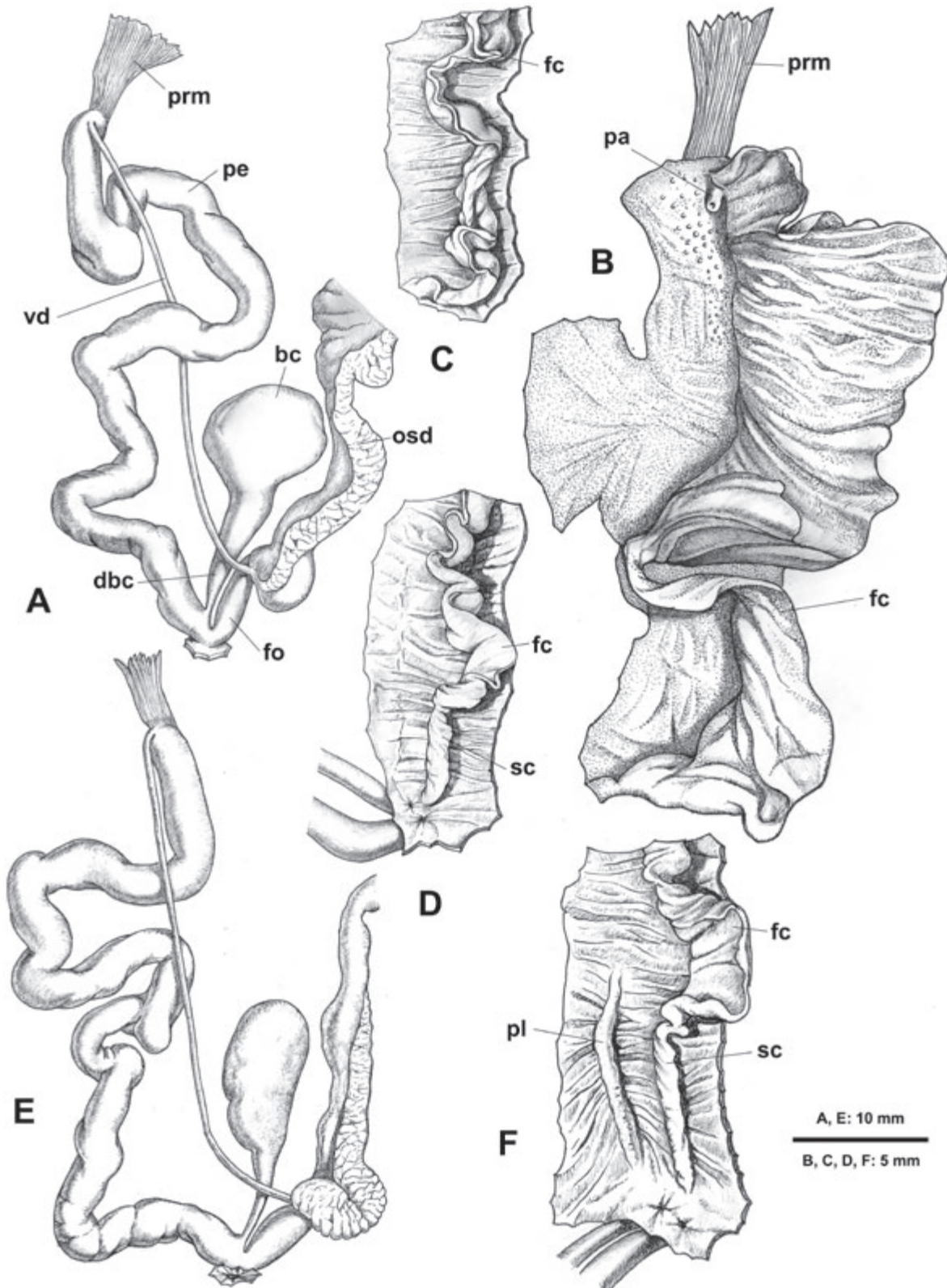
*Digestive system* Intestine forms 5 loops; the last one without rectal caecum.



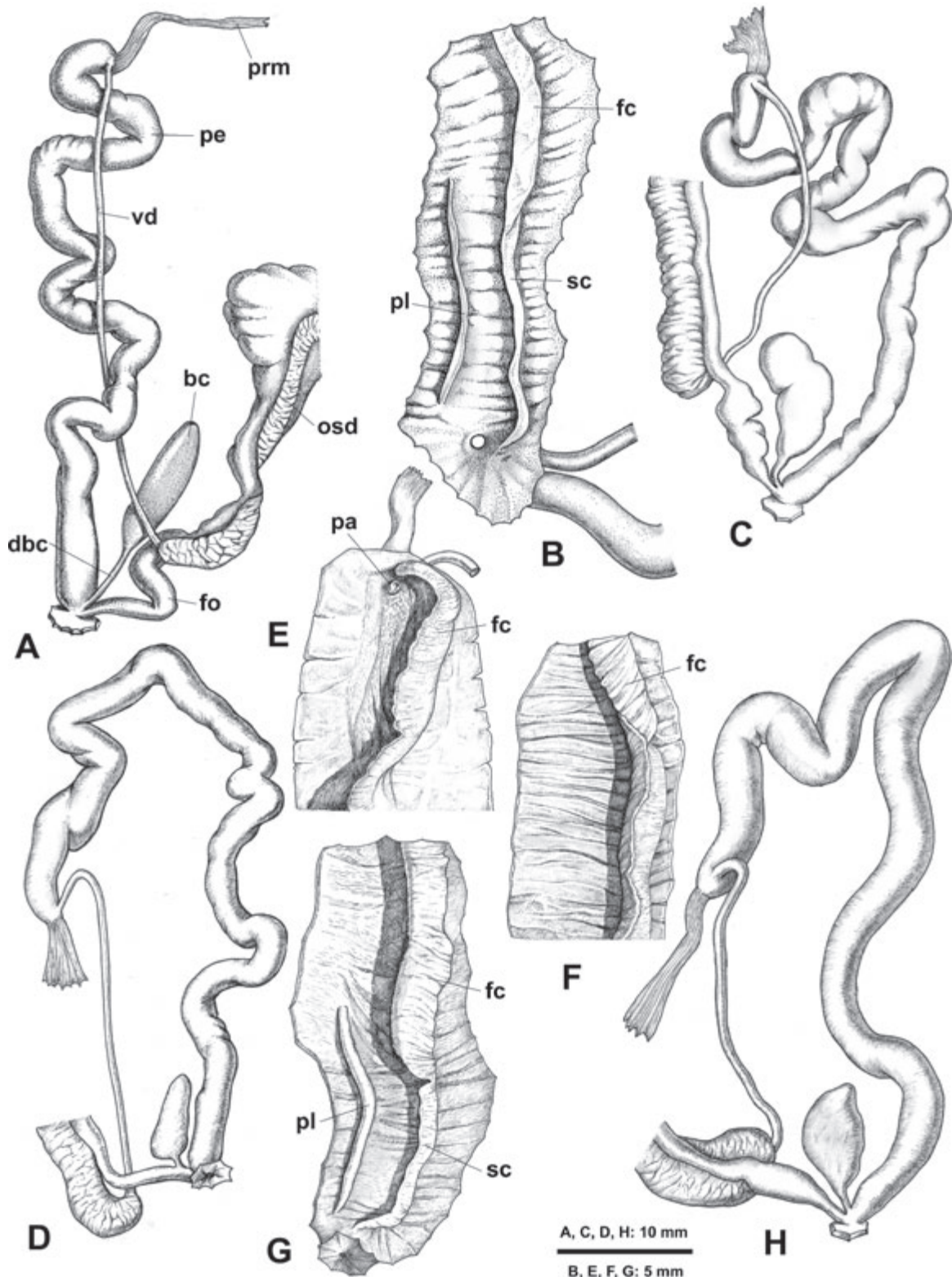
**Figure 1** Living specimens of *Limax cinereoniger*. A: Langeberg, Salem (Ratzeburg, Germany); B: Rakov Škocjan (Postojna, Slovenia); C-D: Lake of Verzegnis (Udine, Italy); E: Cave del Predil, Tarvisio (Udine, Italy); F: near the cave Vranja peč (Bostanj, Slovenia); G: Forcella Moraret near Rifugio Marinelli, Forni Avoltri (Udine, Italy); H: Tárkányi-patak Valley, Bükk Mountains (Miskolc, Hungary); I: Triberg falls (Triberg im Schwarzwald, Germany); J-L: Planda Purcei, Monte San Simeone, Bordano (Udine, Italy).

**Habitat** Based on most collecting sites and according to literature data, this species lives predominantly in forests, especially in non-anthropogenic environments, indifferent to

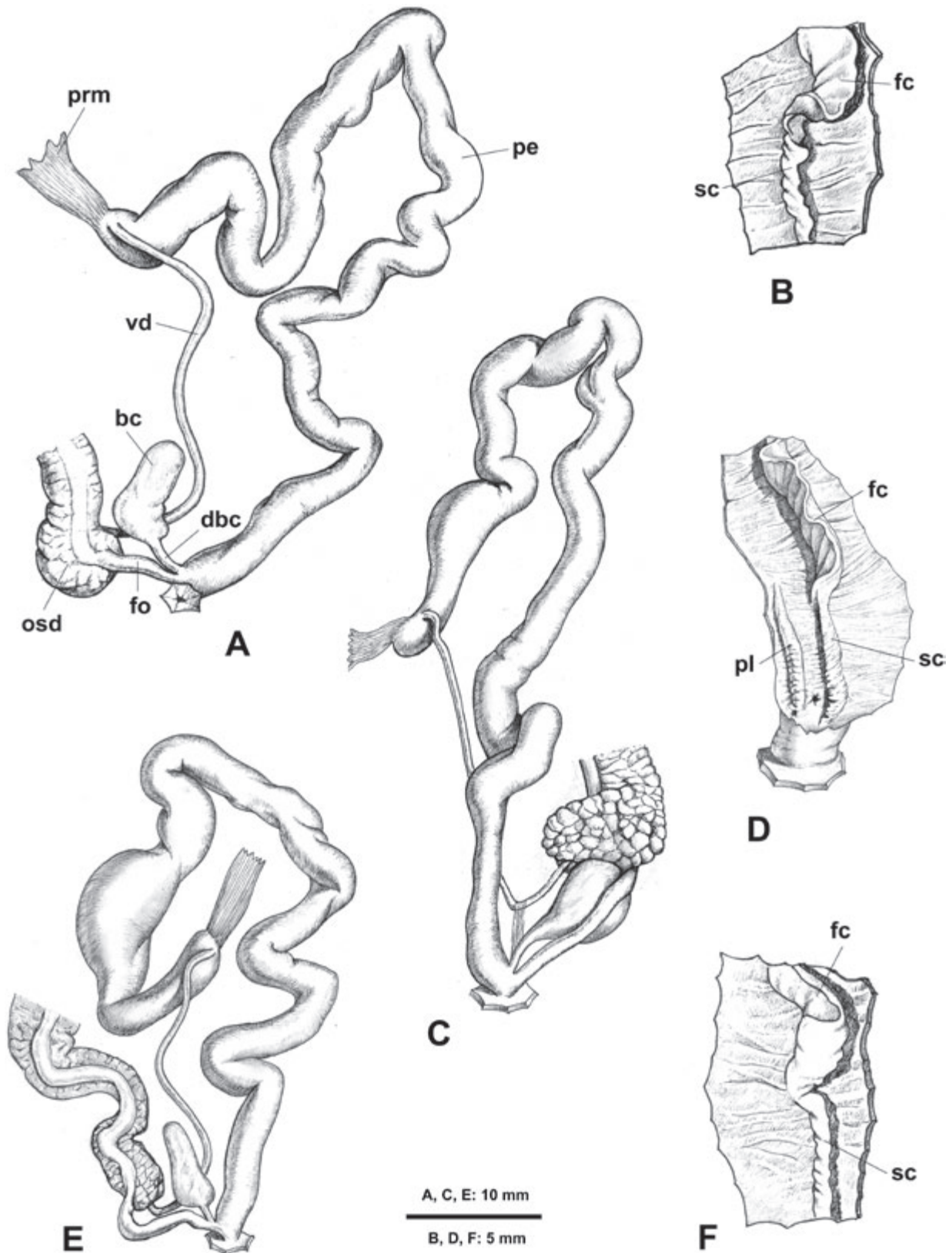
lithology of the substrate. In Central Europe, it is widespread in mature lowland forests; in Italy it is more frequent in deciduous forests of the mountain level or in mixed forests and coniferous



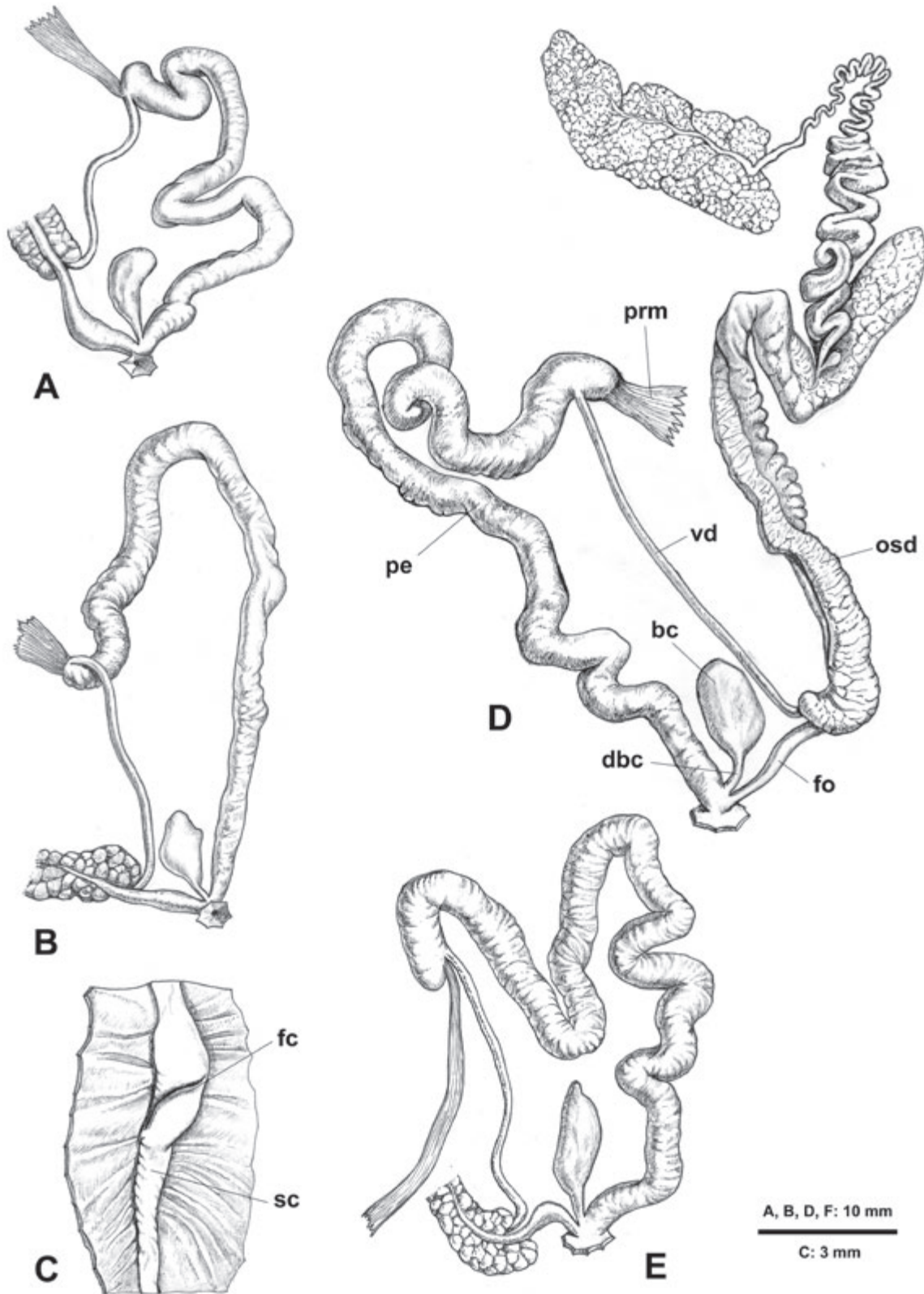
**Figure 2** Genitalia of *Limax cinereoniger* from Germany and Sweden. A, E: outer appearance of genitalia (without proximal portion); B: inner structure of first proximal tract of the penis with the high first crest; C: inner structure of half tract of the penis (between proximal and distal portion) with the lower first crest; D, F: inner structure of last distal tract of the penis with the end of first crest and the beginning of second low crest. Specimens from: Langeberg, Salem, Ratzeburg (Langeberg, Schleswig-Holstein, Germany) (A–D); along the road E22 between Hörby and Linderod, near the fork for Satsrup, Gårdstånga, Skåne (Hörby, Skåne, Sweden) (E–F).



**Figure 3** Genitalia of *Limax cinereoniger* from Austria, France and Switzerland. A, C–D, H: outer appearance of genitalia (without proximal portion); B, G: inner structure of last distal tract of the penis with the end of first crest and the beginning of second low crest; E: inner structure of first proximal tract of the penis with the high first crest; F: inner structure of half tract of the penis (between proximal and distal portion) with the lower first crest. Specimens from: Souterrain 3 au niveau de la Grande Carrière, Le Struthof, Natzwiller (Natzwiller, Bas-Rhin, France) (A–B); Mont-Saxonnex (Mont-Saxonnex, Haute-Savoie, France) (C); Weissenstein, south slope (Oberdord, Solothurn, Switzerland) (D); Raxalpe (Altenberg an der Rax, Steiermark, Austria) (E–H).

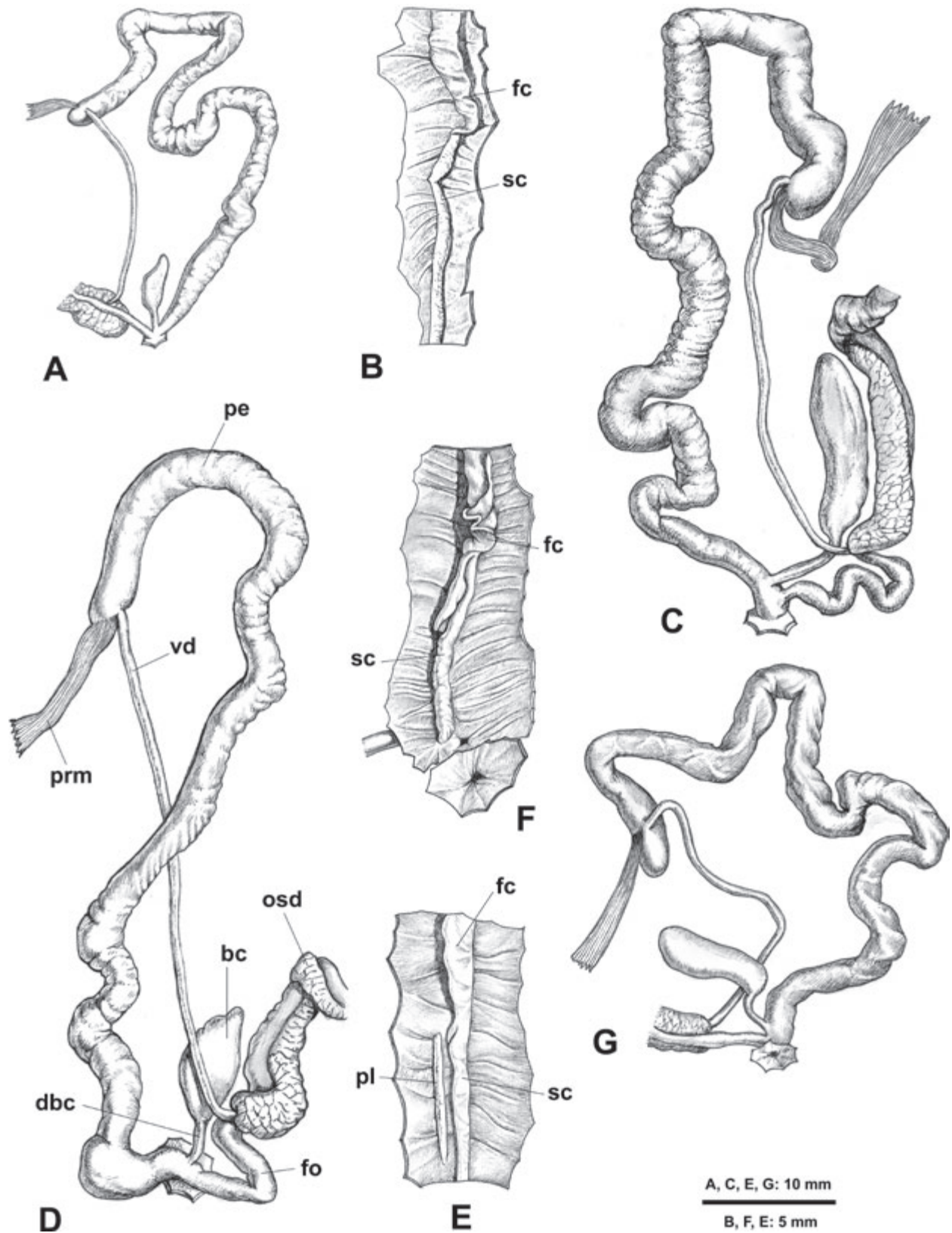


**Figure 4** Genitalia of *Limax cinereoniger* from Croatia, Hungary and Slovenia. A, C, E: outer appearance of genitalia (without proximal portion); B, D, F: inner structure of last distal tract of the penis with the end of first crest and the beginning of second low crest. Specimens from: Rakov Škočjan, Postojna (Cerkljica, Notranjsko-Kraska, Slovenia) (A–B); 1km N from Nagyhuta (Nagyhuta, Sátoraljaújhely, Borsod-Abaúj-Zemplén, Hungary) (C–D); Žudetici (Vižinada, Istarska), Croatia (E–F).



**Figure 5** Genitalia of *Limax cinereoniger* from Trentino-Alto Adige and Veneto, Italy. A–B, E: outer appearance of genitalia (without proximal portion); C: inner structure of last distal tract of the penis with the end of first crest and the beginning of second low crest; D: entire genitalia. Specimens from: Stein, Val di Vizze (Val di Vizze, Bolzano, Trentino-Alto Adige) (A); Unter Nevesalm, Selva dei Molini (Selva dei Molini, Bolzano, Trentino-Alto Adige) (B–C); La Muda, path 546 to Forcella Moschesin (Agordo, Belluno, Veneto) (D); Nagaoni Valley, Dolomiti Bellunesi (Mezzano, Trento, Trentino-Alto Adige) (E).





**Figure 6** Genitalia of *Limax cinereoniger* from Friuli-Venezia Giulia, Italy. A, C, D, G: outer appearance of genitalia (without proximal portion); B, E, F: inner structure of last distal tract of the penis with the end of first crest and the beginning of second low crest. Specimens from: Prescudin, Barcis (Barcis, Pordenone) (A–B); Monte Strabut, slope NE (Tolmezzo, Udine) (C); Planda Purcei, Monte San Simeone (Bordano, Udine), (D–E); Botazzo (San Dorligo della Valle, Trieste) (F–G).

**Table 1** Size (mm) and ratio of the structures of genital tract in the specimens of *Limax cinereoniger* examined, grouped from European countries and Italian regions. Mean  $\pm$  standard deviation and range (in brackets); n: number of specimens.

Country	fo	bc	dbc	vd	p	p/vd	fc	sc	fc/sc	n
Germany	7	13	5	37	76	2.1	74	5	14.8	1
Sweden	6	12	7	38	84	2.2	98	6	16.3	1
Denmark	6.5 $\pm$ 0.7 (6–7)	7 $\pm$ 1.4 (6–8)	2.5 $\pm$ 0.7 (2–3)	27 $\pm$ 9.9 (20–34)	69 $\pm$ 14.1 (59–79)	2.6 $\pm$ 0.4 (2.3–2.9)	70 $\pm$ 4.2 (67–73)	7.5 $\pm$ 2.1 (6–9)	9.7 $\pm$ 2.2 (8.1–11.2)	2
Great Britain	10	6	2	27	56	2.1	54	7	7.7	1
Ireland	7 $\pm$ 1.4 (6–8)	10 $\pm$ 2.8 (8–12)	1.5 $\pm$ 0.7 (1–2)	24 $\pm$ 4.2 (21–27)	54.5 $\pm$ 4.9 (51–58)	2.3 $\pm$ 0.2 (2.1–2.4)	59 $\pm$ 5.7 (55–63)	4 $\pm$ 1.4 (3–5)	15.5 $\pm$ 4.0 (12.6–18.3)	2
France	9 $\pm$ 2.8 (7–11)	11 $\pm$ 0.0 (11–11)	3 $\pm$ 2.8 (1–5)	32.5 $\pm$ 13.4 (23–42)	68 $\pm$ 8.5 (62–74)	2.3 $\pm$ 0.6 (1.8–2.7)	75 $\pm$ 5.7 (71–79)	6.5 $\pm$ 3.5 (4–9)	13.3 $\pm$ 6.3 (8.8–17.7)	2
Switzerland	4 $\pm$ 0.0 (4–4)	6.5 $\pm$ 0.7 (6–7)	1.5 $\pm$ 0.7 (1–2)	27.5 $\pm$ 6.4 (23–32)	72 $\pm$ 2.8 (70–74)	2.7 $\pm$ 0.5 (2.3–3)	79.5 $\pm$ 7.8 (74–85)	5.5 $\pm$ 0.7 (5–6)	14.7 $\pm$ 3.3 (12.3–17)	2
Austria	7.3 $\pm$ 3.2 (5–11)	7.7 $\pm$ 0.6 (7–8)	2.3 $\pm$ 1.2 (1–3)	28 $\pm$ 4.4 (23–31)	86.7 $\pm$ 11.0 (78–99)	3.1 $\pm$ 0.4 (2.7–3.4)	84.7 $\pm$ 9.3 (77–95)	6.7 $\pm$ 1.5 (5–8)	13.2 $\pm$ 3.4 (9.6–16.4)	3
Hungary	7.5 $\pm$ 4.9 (4–11)	7 $\pm$ 4.2 (4–10)	2.5 $\pm$ 0.7 (2–3)	22 $\pm$ 2.8 (20–24)	77.5 $\pm$ 21.9 (62–93)	3.5 $\pm$ 0.5 (3.1–3.8)	96.5 $\pm$ 31.8 (74–119)	4.5 $\pm$ 0.7 (4–5)	21.2 $\pm$ 3.7 (18.5–23.8)	2
Slovenia	7 $\pm$ 2.2 (5–10)	13 $\pm$ 6.6 (6–20)	3 $\pm$ 0.8 (2–4)	35.3 $\pm$ 8.4 (23–42)	111 $\pm$ 44.2 (52–159)	3.1 $\pm$ 0.8 (2.3–4.1)	117 $\pm$ 50.6 (51–174)	9.3 $\pm$ 3.8 (5–14)	13.7 $\pm$ 7.2 (6.4–23.6)	4
Croatia	8.4 $\pm$ 5.9 (3–17)	8.6 $\pm$ 2.9 (5–12)	2.8 $\pm$ 1.6 (1–5)	29.6 $\pm$ 3.2 (26–34)	95 $\pm$ 22.1 (75–130)	3.3 $\pm$ 0.9 (2.2–4.3)	102.8 $\pm$ 19.0 (81–127)	7 $\pm$ 2.0 (5–9)	15.5 $\pm$ 4.1 (9–19)	5
Italy: Trentino- Alto Adige	7 $\pm$ 2.7 (4–12)	7.3 $\pm$ 2.0 (4–10)	2 $\pm$ 1.1 (1–4)	25.8 $\pm$ 7.7 (14–34)	79.6 $\pm$ 26.6 (42–117)	3.1 $\pm$ 0.6 (2–3.8)	78.3 $\pm$ 26.9 (38–114)	5.9 $\pm$ 1.2 (4–7)	13.9 $\pm$ 5.7 (6.3–24.7)	8
Italy: Veneto	10.7 $\pm$ 3.5 (7–15)	7.9 $\pm$ 3.0 (6–14)	2.3 $\pm$ 0.8 (1–3)	27.1 $\pm$ 6.1 (18–34)	78.6 $\pm$ 15.9 (55–102)	3.0 $\pm$ 0.4 (2.4–3.5)	79 $\pm$ 17.2 (54–105)	5.7 $\pm$ 2.0 (3–8)	14.7 $\pm$ 3.9 (10.1–20.5)	7
Italy: Friuli- Venezia Giulia	10.4 $\pm$ 5.2 (4–20)	8.4 $\pm$ 3.3 (5–16)	2.9 $\pm$ 1.5 (1–7)	28.5 $\pm$ 6.5 (19–42)	74.8 $\pm$ 11.8 (61–103)	2.8 $\pm$ 0.7 (1.8–4.1)	73.3 $\pm$ 15.5 (53–110)	7.8 $\pm$ 2.4 (2–12)	9.4 $\pm$ 3.4 (5.3–15.7)	12
MIN	3	4	1	14	42	1.8	38	2	5.3	3
MAX	20	20	7	42	159	4.3	174	14	24.7	20

forests of the alpine plain, where exceptionally it reaches more than 2000 meters above sea level.

**Distribution** (Fig. 8) In the literature the species is known from almost the whole of Europe (Welter-Schultes, 2012; Bank & Neubert, 2017), but in many countries accurate anatomical investigations and genetic studies are still missing. Certainly, it is present throughout central Europe and, in addition to the countries where the species has been confirmed after genetic checks, such as Great Britain, Ireland, The Netherlands, France, Switzerland and Germany (Nitz *et al.*, 2009; Rowson *et al.*, 2014a), its presence is also confirmed, based on our anatomical data, from Sweden, Denmark, Austria, Hungary, Slovenia, Croatia and Italy (limited to the north-eastern areas). For other European countries, especially for the southern and eastern ones, such as Spain

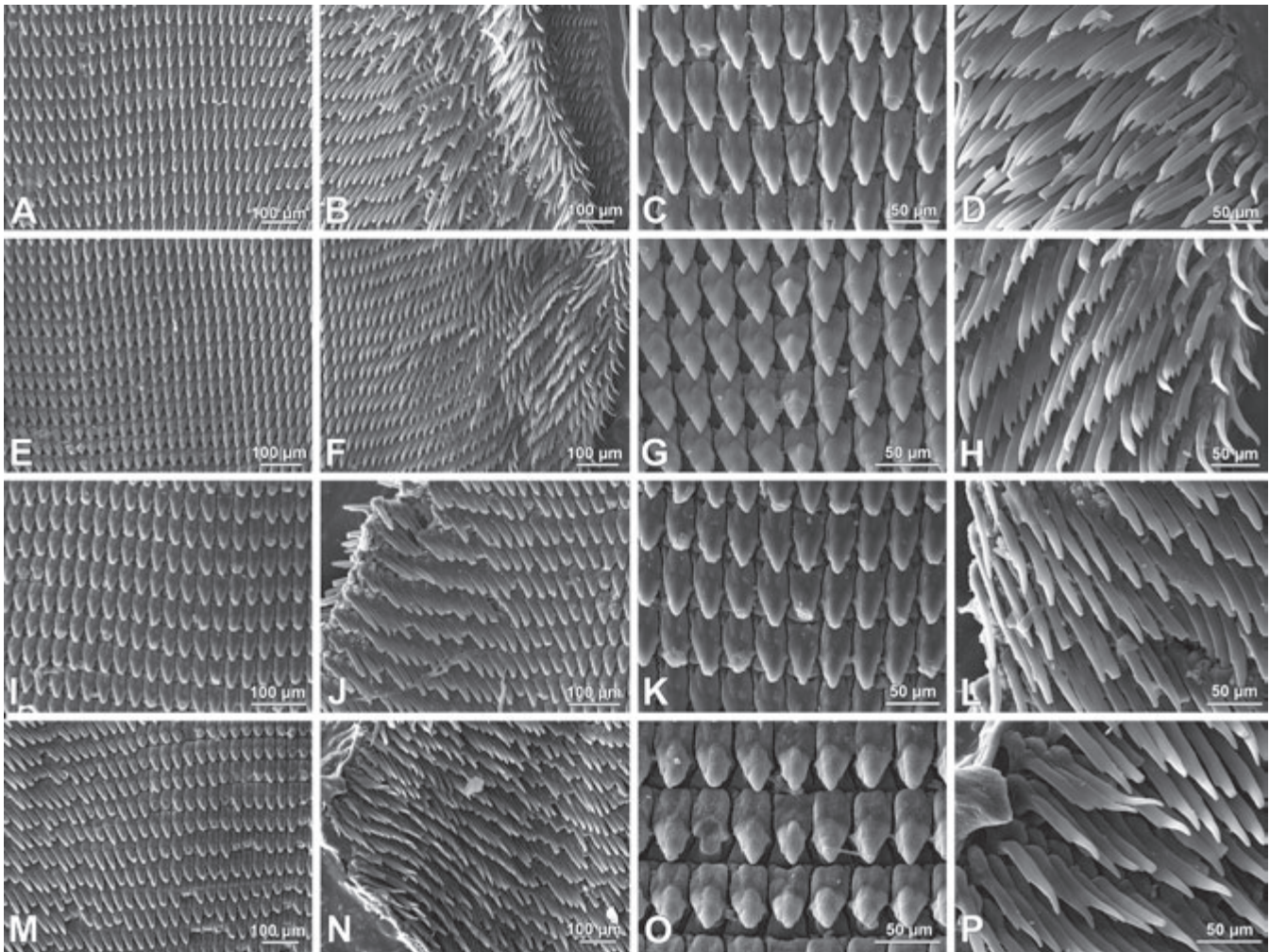
(Cadeval & Orozco, 2016), the southern Balkan countries (Wiktor, 1983, 1996; Fehér & Eröss, 2009), Russia and Ukraine (Likharev & Wiktor, 1980; Gural-Sverlova & Gural, 2012), the literature records must be verified, even if they contain anatomical illustrations.

## DISCUSSION

### Diagnostic characters of species belonging to the genus *Limax*

In the literature, *Limax* species are often distinguished by body colour and penis length; however, both these characters are very variable and do not allow the safe determination of different taxa.

The body colouring permits some groups of species to be distinguished and while colouration of the dorsum is usually very variable that

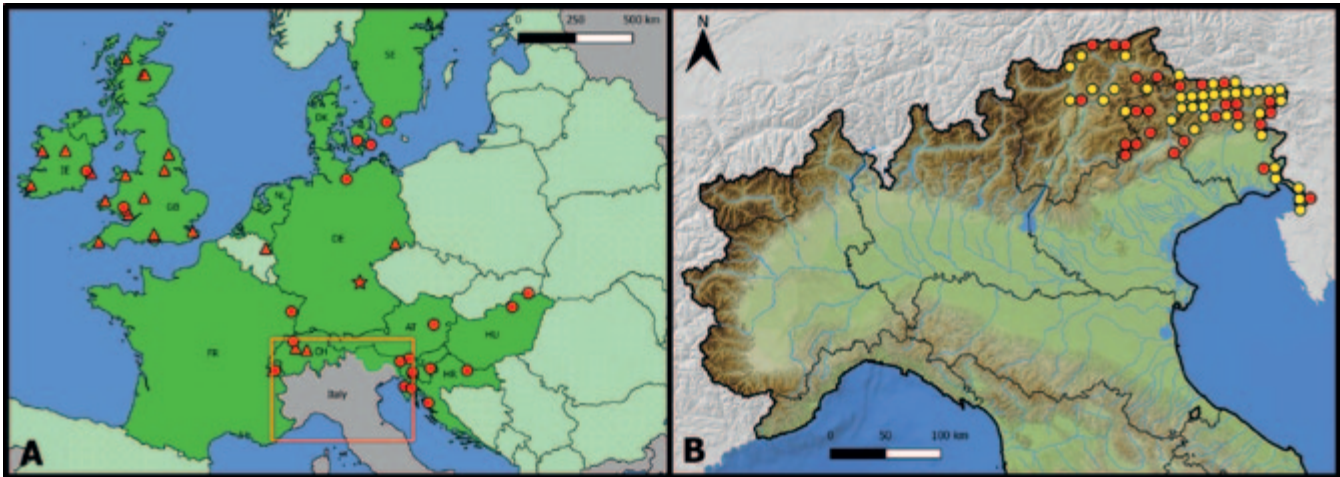


**Figure 7** Radula of *Limax cinereoniger* from Germany (A–D), Great Britain (E–H), Switzerland (I–L) and Italy (M–P). A, E, I, M: central and lateral teeth; B, F, J, N: marginal teeth; C, G, K, O: enlargement of central and lateral teeth; D, H, L, P: enlargement of marginal teeth. Specimens from: Langeberg, Salem, Ratzeburg (Langeberg, Schleswig-Holstein) (A–D), Brecon Beacons National Park (Powy, Wales) (E–H), Weissenstein, south slope (Oberdord, Solothurn) (I–L), Near Staboli Prà di Lovena, Illeggio, Val di Lunze (Tolmezzo, Udine, Friuli-Venezia Giulia) (M–P).

of the sole is much more consistent. *L. maximus* Linnaeus, 1758, *L. redii* Gerhardt, 1933, and *L. ianninii* Giusti, 1973, present a unicoloured white sole while *L. corsicus* Moquin-Tandon, 1855, has a sole with orange side portions, although this is visible only in living specimens. *L. erythrus* Bourguignat, 1864, and *L. alpinus* Férussac, 1822, have a sole with dark side portions where the blackish colour occupies only the edge of the sole (not the entire side portions) whereas in *L. cinereoniger*, *L. subalpinus* Lessona, 1880, and *L. dacampi* Menegazzi, 1854, (Fig. 9) the blackish colouration occupies the entire side portions. The colour of sole is consistent in most with the exception of some specimens of species with a three-banded sole that may have an entirely

pale sole (e.g., *L. dacampi*) (Giusti, 1973a; Falkner, 1990; Turner *et al.*, 1998; Falkner *et al.*, 2002; Nitz *et al.*, 2010; Boschi, 2011; Welter-Schultes, 2012; Manganelli *et al.*, 2014; unpublished personal data).

Penis length also permits some groups of species to be distinguished: taxa having a short penis (less than 50mm in preserved specimens), like *L. maximus*, *L. alpinus* and *L. subalpinus*; taxa having a long penis (50–160mm in preserved specimens), as in *L. cinereoniger* and *L. dacampi*; or taxa having a very long penis (more than 160mm in preserved specimens), as in *L. redii* and *L. erythrus*. However, the penis length is not a reliable diagnostic character because it can be variable within species; in *L. erythrus* for example, its range is 213–615mm.



**Figure 8** Distribution of *Limax cinereoniger* in Europe (A), and in Italy (B). Red star: type locality where specimens have been confirmed by genetic data (Wolf, 1803; Nitz *et al.*, 2009); red triangle: localities where specimens have been confirmed by genetic data (Nitz *et al.*, 2009; Rowson *et al.*, 2014a); red dots: localities where specimens have been confirmed anatomically (present study); yellow dots: other Italian localities where specimens have been determined without fine anatomical studies (young specimens with immature genitalia, external morphological characters or by verified literature; De Mattia, 2003; Dalfredo, 2007; Welter-Schultes, 2013; personal unpublished data); dark green: countries from where the taxon has been confirmed by anatomical or genetic data (Nitz *et al.*, 2009; Rowson *et al.*, 2014a; present study); light green: countries from where the taxon has been reported based on literature data (summarised by Bank & Ramos, 2017) but not confirmed by detailed anatomical or genetic data. The symbols in the Italian map are in the centre of the 10km squares of UTM grid. Cartography from: ARPA Piemonte – Europe hillshade WM (Web Map Tile Service WMTS) and Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) – Digital Terrain Model – 75 meters (DEM 75 – dataset); administrative boundaries from: Thematic Mapping.org by Bjorn Sandvik – World Borders Dataset, and ISTAT – administrative boundaries 2017 in not generalised version (ISTAT, 2017); hydrography from The National Geoportal – hydrographical network and inland water bodies (Web Feature Services WFS).

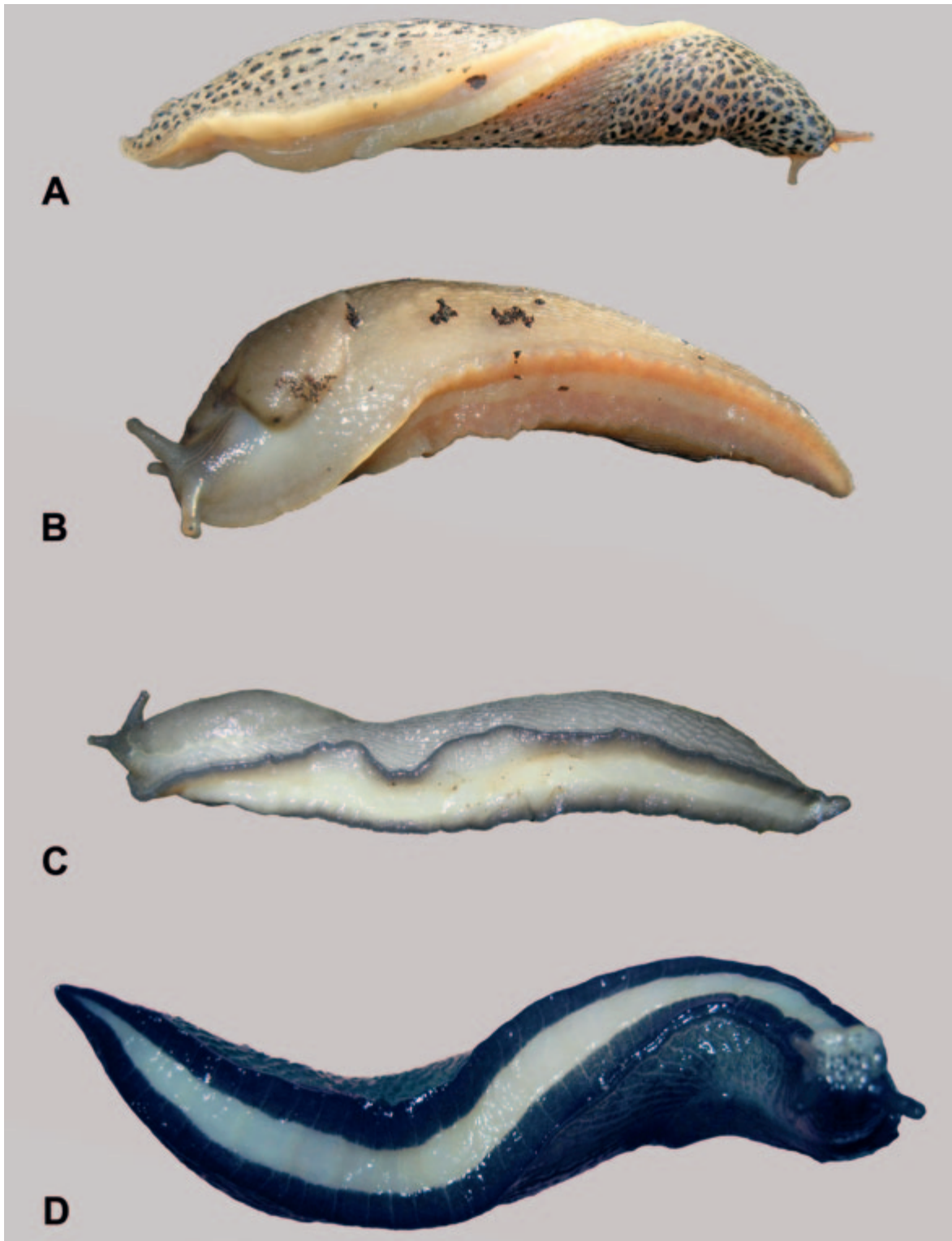
Also in *L. corsicus* its variability is very high, but today this taxon is considered a species complex, separated into several other distinct taxa, such as *L. ciminensis* Pollonera, 1890, *L. giustii* Falkner & Nitz, 2010, *L. ilvensis* Falkner & Nitz, 2010, *L. senensis* Pollonera, 1890, *L. vizzavonensis* Falkner & Nitz, 2010, and *L. wolterstorffi* (Simroth, 1900) (Falkner *et al.*, 2002; Nitz *et al.*, 2010; Manganelli *et al.*, 2014; Bank & Neubert, 2017).

This set of characteristics, such as the colour of the dorsum, the colour of the sole and the length of the penis, allow the determination to be directed or narrowed, but not the certain differentiation of the species within each group. A further step to recognise the different taxa can be done by studying the inner structures of penis, a diagnostic character used in a few publications, frequently without a detailed analysis and without a wide comparison among different species (Giusti & Mazzini, 1971; Giusti, 1973a, 1973b; Rähle, 1981; Falkner & Niederhöfer, 2008; Nitz *et al.*, 2009). Based on this anatomical character, the different species are almost always reliably recognisable.

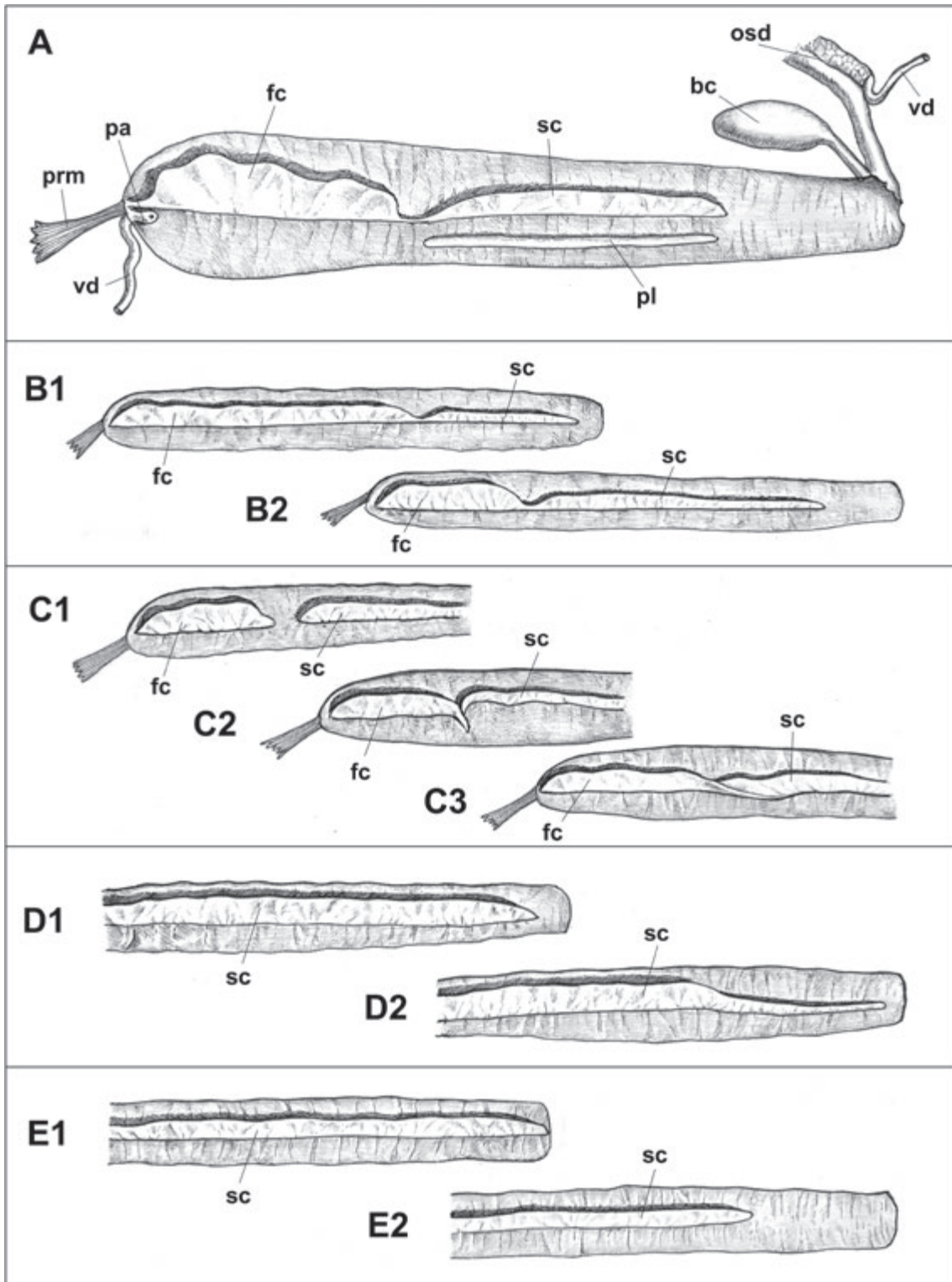
#### Inner structures of penis in the genus *Limax* (Fig. 10)

In the different *Limax* species, the inner wall of the penis presents, with only a few exceptions, three different primary structures: (1) a first crest, usually high, longitudinally extended from the apex (proximal end of the penis) for some length; (2) a second crest, lower, which follows the first one and occupies longitudinally the next section towards the atrium; (3) a low longitudinal pleat, which starts on the inner wall of penis at the beginning of second crest, running parallel to the crest (Fig. 10 A).

Some elements allow a diagnostic value: (i) the ratio between the two crests' lengths; (ii) the morphology of the contact point between the two crests; (iii) the height of the second crest in the proximal (close to the insertion of vas deferens) and the distal (close to the genital atrium) tracts of penis; (iv) the length and the end of second crest with respect to the end of penis (towards the genital atrium); (v) the length and the end of the pleat, opposite to the second crest, with



**Figure 9** Examples of sole colouring in some species of *Limax*. A: unicolorous white sole (*L. maximus*); B: tripartite sole, with orange lateral portions (*L. corsicus*); C: tripartite sole, where the blackish colour occupies only the edge of the sole (*L. erythrus*); D: tripartite sole, where the blackish colour occupies the entire lateral portions (*L. cinereoniger*). Specimens from: Lazetta (Mezzenile, TO, Italy), 32T LR7416, S. Birindelli, M. Marietta & M. Poggio leg. 27/10/2012 (A); Pian Grande (Marliana, PT, Italy), 32T PP4468, S. Cianfanelli & M. Calcagno leg. 25/04/2009 (B); Case Favetto (Rueglio, TO, Italy), 32T MR0436, S. Birindelli, A. Cavallero, L. Iorio & E. Magnetti leg. 06/08/2011 (C); Forcella Moraret near Rifugio Marinelli (Forni Avoltri, UD, Italy), 33T UM3862, S. Cianfanelli, M. Calcagno, G. Manganelli & L. Manganelli leg. 28/08/2009 (D).



**Figure 10** Schematic picture of main distinctive characters and appearance of inner structure of the penis in the genus *Limax*. A: main characters of inner structure; B: appearance in the length of first and second crest: first crest longer than second crest (B1) or shorter (B2); C: appearance in the tract between the first and the second crest: separate crests (C1), first crest that ends laterally (on the right) from the second one (C2); first and second crest merge into each other (C3); D: height of second crest in the distal portion of the penis: crest rather high also in the last tract, close to the genital atrium (D1), crest lower in the last tract, close to the genital atrium (D2); E: end of second crest relative to the end of the distal penis: crest ending at the end of the penis (E1), crest ending proximal of the end of penis (E2).

respect to the end of penis (towards the genital atrium). Moreover, in some species, other peculiar characters are present, such as small papillae or a callus.

The first crest can be longer than the second (Fig. 10 B1) or shorter (Fig. 10 B2). The contact point between the two crests, in addition to the different height (low or high) and the position occupied with respect to the penis length, related to the different growth of the two crests, shows a different morphology (Fig. 10 C). In *L. subalpinus* the two crests are separated by a callous pad (Fig. 10 C1), placed in the separation area but in the opposite wall of the penis; however the crests are commonly contiguous, and often the first crest ends to the right while the second begins on its left side, just before the end of first crest, in the proximal portion of the penis (Fig. 10 C2). In some cases, the end of first crest and the beginning of second crest are parallel to each other (Fig. 10 C3). The second crest is generally high in its first section, then it can remain high all along its length (Fig. 10 D1), or appear very low in its second portion, with a similar appearance to the pleat (Fig. 10 D2); it can continue up to the atrium (Fig. 10 E1), or stop well before the end of the penis (Fig. 10 E2). The pleat, which may be more or less developed, usually follows the same development of second crest, so it can proceed to the proximity of the atrium or it can end before. Finally, a single papilla may be present at the outlet of the vas deferens and more or less developed small papillae can cover the convex side of crests and/or the inner wall of penis.

#### Diagnostic characters in *Limax cinereoniger* (Fig. 11)

In addition to the sole colour (blackish lateral bands), relatively long penis (42–159mm) and the short vas deferens, compared to the penis length (14–42mm; ratio penis/vas deferens: from 1.8 to 4.3), the main character to distinguish this species is represented by the length of first crest, significantly greater than that of the second crest (ratio between the length of first and that of second crest: from 5.3 to 24.7) (Figs 10 B1, 11 A), while in almost all other *Limax* species the first crest is always shorter than the second one. In *L. cinereoniger* the contact point between the first and the second crest is situated near the distal end of the penis (close to the genital atrium), and it is variable in height in different specimens; the

first crest, still quite high in its terminal portion, lowers abruptly and ends to the right, while the second one begins on its left; the two crests are adjacent usually only for a short portion. The second crest is low and short and reaches the atrium. The pleat parallel to the second crest is low and short, sometimes very weak, barely visible.

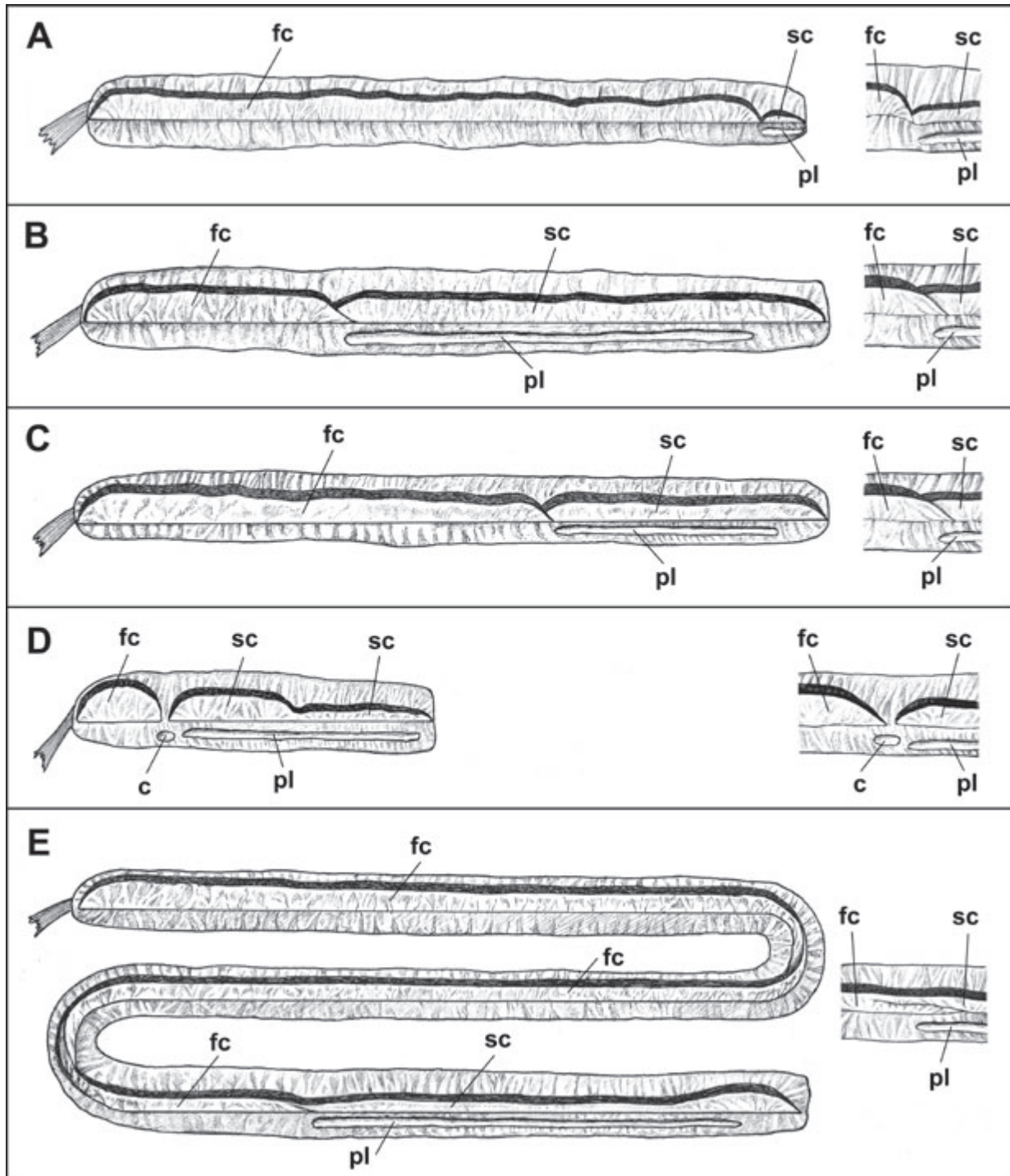
The following characters can distinguish the other central-European and Italian species that demonstrate a similar sole colouration (entire blackish lateral bands):

*L. dacampi* (56 sp examined, from Valle d'Aosta, Piedmont, Friuli-Venezia Giulia and Liguria): penis equally long (42–158mm) as in *L. cinereoniger*, but second crest longer than the first one (*L. dacampi dacampi*, ratio between the first and the second crest: from 0.23 to 0.9; Fig. 11 B), or quite similar length (*L. dacampi cruentus* Lessona, 1880, ratio between the first and the second crest: from 0.8 to 2.8; Fig. 11 C); crests partially overlying in the contact area, where the end of first crest runs parallel to the beginning of second crest (on its left) for a short portion; the second crest reaches the atrium and is still quite high just before its end.

*L. subalpinus* (26 sp examined, from Piedmont and Liguria; Fig. 11 D): penis on average shorter (24–72mm), with first crest significantly shorter than the second one (ratio between the first and the second crest: from 0.14 to 0.50); crests usually separated, with a large callus in the inner wall of penis, opposite to the separation zone; second crest usually high in the first portion and low in the second portion, where it assumes the appearance of a pleat.

*L. sp. 2* (after Manganelli *et al.*, 1995; "*L. callichrous*" after Giusti & Mazzini, 1971) (21 sp examined, from Piedmont, Emilia-Romagna, Liguria and Tuscany; Fig. 11 E): very long penis (174–405mm); first crest as long or longer than the second one (ratio between the first and the second crest usually lower to that of *L. cinereoniger*: from 0.7 to 5.8); contact area just visible, sometimes unclear; the second crest reaches the atrium and is quite high just before its end.

Outside of central-Europe and Italy, two other taxa with a sole with blackish lateral bands (occupying the entire side portions) have been described: *L. wohlberedti* Simroth, 1900, from the Balkans and *L. bielzii* Siebert, 1873, from the Carpathians. The first species has a very short



**Figure 11** Schematic picture of penis appearance with main distinctive inner structures, crests and pleat, in lateral view (left), and detail of contact point between first and second crest, in top view (right) in *Limax cinereoniger* and in other central-European and Italian species belonging to the genus *Limax*, showing a similar sole colouration (entire blackish lateral bands). A: *L. cinereoniger*: quite long penis, very short and lower second crest; B: *L. dacampi dacampi*: quite long penis, first crest shorter than the second crest, contact point where the first crest runs parallel to the beginning of the second one; C: *L. dacampi cruentus*: quite long penis, first crest longer than the second crest, contact point where the first crest runs parallel to the beginning of the second one; D: *L. subalpinus*: penis short, crests separated, with presence of callus in the inner wall of the penis, second crest low in the distal portion of the penis; E: *L. sp. 2*: very long penis, first crest quite similar or longer than the second one, contact area just visible, second crest higher before its end rather than in central tract. Proportional dimensions of the penis and the crests based on the medium size of studied specimens.



penis, as long as the vas deferens (Wiktor, 1996, fig. 103), while the penis of second one is similar in appearance and dimensions to that of *L. cinereoniger* (Wiktor, 1973, fig. 98). However, no recent, focused studies have been undertaken to clarify the taxonomic status of these two species and their relationship to *L. cinereoniger*.

#### Assignment of the examined material to *Limax cinereoniger*

The studied materials (see Appendix) come from a German site located about 600km further north of the type locality of the species and from elsewhere in Europe, i.e. Sweden, Denmark, Great Britain, Ireland, Austria, Hungary, Slovenia and Croatia. In all these countries, only a single autochthonous *Limax* with a three-banded sole, is known (Wiktor & Szigethy, 1983; Wiktor, 1996; Fehér & Gubányi, 2001; Vaupotič & Velkovich, 2003; Waldén, 2007; Štamol, 2010; Rowson *et al.*, 2014b; Wiese, 2014; Fischer, 2015; Bank & Neubert, 2017; Skipper, 2017). Also for two other countries, at least a part of the examined materials was collected in areas where only a single taxon with a three-banded sole is present, as for mainland France or northern Switzerland (Turner *et al.*, 1998; Falkner *et al.*, 2002; Boschi, 2011).

Genetic data from European populations, such as from Great Britain, Ireland, France, Switzerland, The Netherlands, Germany, including the type locality (Oberkrumbach), identify only one cluster, belonging to *Limax cinereoniger* (Nitz *et al.*, 2009; Rowson *et al.*, 2014a). This cluster shows affinity only with some undetermined taxa from France and Italy (Rowson *et al.*, 2014a). Among other taxa genetically examined, *Limax alpinus* Férussac, 1822, (an older synonym of *Limax sarnensis* Heim & Nitz, 2009, according to Brandstetter, 2011) and *Limax* cf. *dacampi* (Rowson *et al.*, 2014a, 2014b, instead a taxon probably attributable to *Limax* sp. 2 of Manganelli *et al.*, 1995, for its very long penis) are positioned in clearly separated clusters (Nitz *et al.*, 2009; Rowson *et al.*, 2014a).

So, we are certain that all material examined by us and presented here belongs to *L. cinereoniger* for the following reasons: all the specimens come from areas where not related taxa has been reported; they correspond morphologically and anatomically to this taxon; they include populations close to those genetically examined and

attributed to the species, such as those from northern Switzerland (Nitz *et al.*, 2009).

#### *Limax cinereoniger* in Italy

Many taxa, with a colouration similar to that of *L. cinereoniger*, were cited or described for Italy, but all have recently been considered to belong to other species (Manganelli *et al.*, 1995; Bank & Neubert, 2017). Specimens from north-eastern Italy, similar in appearance (body black in colour) and in anatomy (length of the penis) to *L. cinereoniger*, were attributed, with doubt, to *L. bielzi* Siebert, 1873, a species with an eastern European distribution (Boato *et al.*, 1989; Dalfreddo *et al.*, 2000; De Mattia, 2003). Indeed, the external genitalia seems to correspond to that already known in the literature for *L. bielzi*, which is quite similar to that of *L. cinereoniger*. Whilst the inner structure of the penis of *L. bielzi* is not known, the few elements published on the inner structure of British specimens of *L. cinereoniger* ("internally it bears a prominent fold, double at its origin"; Quick, 1960), suggests the presence of two elements, crest and pleat, at the proximal end of the penis, a singular character not found in any *Limax* species studied so far. This judgment, in the absence of a clarifying image, probably needs a different interpretation; for example, the presence of a double structure at the proximal end of the penis, could result from the folding of the same first crest near the insertion points of the retractor muscle and the vas deferens. Anyway, even if the term "proximal" could be interpreted as "near the genital atrium", the two folds would be low and not prominent.

Moreover, the examination of a specimen from Great Britain allowed us to check the internal structure of the penis, where a single crest is present, running along the penis for almost all its length. This structure is the same in specimens from Germany and from other European countries examined, including those from north-eastern Italy.

Therefore, in conclusion, while the presence of *L. cinereoniger* in Italy is confirmed in the north-eastern area of this country (Fig. 8 B), the taxonomic status of *L. bielzi* is not clear (Likharev & Wiktor, 1980). *L. bielzi* could become a junior synonym of *L. cinereoniger*; however, a comparison with topotypic specimens from the Carpathians will be necessary to clarify its taxonomic position.

## CONCLUSIONS

This anatomical approach to the study of the slugs belonging to the genus *Limax*, based on the inner anatomy of the penis, has permitted the morphological differentiation of species and has clarified the diagnostic characters of *L. cinereoniger*, giving concordant results to those already obtained by genetic analysis. However, to completely resolve the taxonomic problems of this group more collaboration is required using an integrated approach to compare anatomical analysis (inner structures of penis) with genetic data, especially for the populations coming from the type localities of many of the taxa described in older literature.

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## APPENDIX

### List of localities and examined material

The collection data are listed as follows: collecting sites, altitude (meters above sea level); municipality (except for Great Britain and Ireland), province and regions in parentheses; UTM coordinates (ED 50), collector(s) and collection date; number of specimens and abbreviations of the Italian collections where they are deposited in parentheses. For the Italian territory, the names of localities were taken from the Italian official cartography, produced by I.G.M.I. (Istituto Geografico Militare Italiano), and the

UTM coordinates were taken from the same source or detected by GPS.

### Germany

- Langeberg, Salem, Ratzeburg, 55m a.s.l. (Langeberg, Schleswig-Holstein), 32U PE2047, M. & E. Bodon leg. 25/07/2011 (1 sp, MZUF GC/52424, stub MB/100; Figs 1 A, 2 A–D).

### Sweden

- Along the road E22 between Hörby and Linderöd, near the street branching off to Satsrup, Gårdstånga, Skåne, 150m a.s.l. (Hörby, Skåne), 33U VB2095, M. & E. Bodon leg. 11/07/2011 (1 sp, MZUF GC/ 53740; Fig 2 E–F).

### Denmark

- Between Karrebaeksmind and Naestev near Karrebaekstord, Sjaelland island, 15m a.s.l. (Naestred, Vestsjaelland, Sjaelland), 32U PG7021, S. Cianfanelli & M. Calcagno leg. 01/08/2000 (1 sp, MZUF GC/52620).

- Møns Klint Storeklint, Møn Island, 120m a.s.l. (Vordingborg, Vestsjaelland, Sjaelland), 33U LF4394, S. Cianfanelli & M. Calcagno leg. 01/08/2000 (1 sp, MZUF GC/52621).

### Great Britain

- Pont ar Daf Car Park on the road 470, Brecon Beacons National Park, 450m a.s.l. (Powys, Wales), 30U VC6746, S. Cianfanelli & M. Calcagno leg. 26/08/1994 (1 sp, MZUF GC/52635, stub MB/101).

### Ireland

- Laragh Glendalough, 170m a.s.l. (Leinster, Wicklow) 29U PU8176, S. Cianfanelli & M. Calcagno leg. 22/07/1998 (1 sp, MZUF GC/52639).

### France

- Souterrain 3 au niveau de la Grande Carrière, Le Struthof, Natzwiller, 830m a.s.l. (Natzwiller, Bas-Rhin), 32U LU7167, M. & E. Bodon leg. 28/12/2011 (1 sp, MZUF GC/53742; Fig. 3 A–B).

- Mont-Saxonnex, 900m a.s.l. (Mont-Saxonnex, Haute-Savoie), 32T LS00, M. Bodon leg. 23/08/1991 (1 sp, MZUF GC/53741; Fig. 3 C).

### Switzerland

- Weissenstein, south slope, 1000m a.s.l.

(Oberdord, Solothurn), 32T LT83, M. Bodon & G. Manganelli leg. 12/06/1996 (2 sp, MZUF GC/53236, stub MB/103; Fig. 3 D).

#### Austria

- Barent Valley, Freistritz im Rosental, 900m a.s.l. (Klagenfurth, Kärnten), 33T VM3549, I. Niero leg. 24/06/2005 (1 sp, INC).  
 - Raxalpe (Altenberg an der Rax, Steiermark), 33T WN58, collector unknown 05/05/1968 (2 sp, FGC; Fig. 3 E–H).

#### Hungary

- 1km N from Nagyhuta, 320m a.s.l. (Nagyhuta, Sátoraljaújhely, Borsod-Abaúj-Zemplén), 34U EU3565, S. Cianfanelli & M. Calcagno leg. 06/07/2016 (1 sp, MZUF GC/52086; Fig. 4 C–D).  
 - Tárkányi-patak Valley, Bükk Mountain, 380m a.s.l. (Felsőtárkány, Eger, Heves), 34U DU6017, S. Cianfanelli & M. Calcagno leg. 05/07/2016 (1 sp, MZUF GC/52084; Fig. 1 H).

#### Slovenia

- River bank of Soča River between Malnig and Cerza, about 5km East of Bovec, 420m a.s.l. (Bovec, Gorizia) 33T UM9332, S. Cianfanelli & M. Calcagno leg. 23/07/1996 (1 sp, MZUF GC/52363).  
 - Ljubljana springs, 295m a.s.l. (Vrhnik, Osrednjeslovenska), 33T VL4589, I. Niero leg. 17/07/1999 (1 sp, INC).  
 - Near the cave Vranja peč, Bostanj, 250m a.s.l. (Sevnica, Spodnjeposavska), 33T WL29, M. Bodon leg. 14/06/1985 (2 sp, MZUF GC/53743; Fig. 1 F).  
 - Rakov Škocjan, Postojna, 550m a.s.l. (Cerknica, Notranjsko-Kraska), 33T VL47, M. Bodon leg. 18/06/1985 (1 sp, FGC; Figs 1 B, 4 A–B).

#### Croatia

- Žudetici, 150m a.s.l. (Vižinada, Istarska), 33T VL02, M. Bodon leg. 18/06/1985 (2 sp, MZUF GC/53744; Fig. 4 E–F).  
 - Road Z 5047 from Vranja to Vela Ucka, 870m a.s.l. (Pazin, Istarska), 33T VL3519, I. Niero leg. 26/07/2015 (1 sp, INC).  
 - 1km from crossroad D 52 to Gornj Babinotok, 790m a.s.l. (Otocac, Lika-Senj), 33T WK0549, Niero leg. 27/07/2015 (1 sp, INC).  
 - 2.4km E from Loncarica, road E661 to Virovitica, 250m a.s.l. (Virovitica, Virovitičko-podravsk), 33T XL8069, S. Cianfanelli & M. Calcagno leg. 09/07/2016 (1 sp, MZUF GC/52087).

#### Italy

- Ormanico, 1040m a.s.l. (Fiera di Primiero, Trento, Trentino-Alto Adige), 32T QS1917, I. Niero leg. 07/08/2001 (1 sp, INC).  
 - Nagaoni Valley, Dolomiti Bellunesi, 1100m a.s.l. (Mezzano, Trento, Trentino-Alto Adige), 32T QS2113, C. Dalfreddo leg. 29/06/1997 (1 sp, CDC; Fig. 5 E).  
 - Aussersage water mill, Velasio, 1300m a.s.l. (San Genesio Atesino, Bolzano, Trentino-Alto Adige), 32T PS7758, I. Niero leg. 24/05/2002 (1 sp, INC).  
 - Left of Isarco River, Fleres Valley, 1260m a.s.l. (Brennero, Bolzano, Trentino-Alto Adige), 32T PT8401, I. Niero leg. 13/07/2001 (1 sp, INC).  
 - Stein, Val di Vizze, 1600m a.s.l. (Val di Vizze, Bolzano, Trentino-Alto Adige), 32T QT0006, M. Bodon leg. 17/07/1995 (1 sp, MZUF GC/53745; Fig. 5 A).  
 - Unter Nevesalm, Selva dei Molini, 1900m a.s.l. (Selva dei Molini, Bolzano, Trentino-Alto Adige), 32T QT1103, M. Bodon leg. 19/07/1995 (1 sp, MZUF GC/53746; Fig. 5 B–C).  
 - San Vigilio, 1200m a.s.l. (Marebbe, Bolzano, Trentino-Alto Adige), 32T QS2475, G. Nardi & A. Braccia leg. 24/06/2007 (1 sp, GNC).  
 - “Parco Natura”, Dobbiaco, 1250m a.s.l. (Dobbiaco, Bolzano, Trentino-Alto Adige), 33T TM8877, I. Niero leg. 04/07/2016 (1 sp, INC).  
 - Malga Le Prese, Boalone Valley, Dolomiti Bellunesi, 1550m a.s.l. (Sovramonte, Belluno, Veneto), 32T QS1607, I. Niero leg. 26/05/2011 (1 sp, INC).  
 - Malga Ciapela, Marmolada, 1450m a.s.l. (Rocca Pietore, Belluno, Veneto), 32T QS2345, G. Nardi & A. Braccia leg. 12/06/2005 (1 sp, GNC).  
 - Palafavera, upper Zoldana Valley, 1600m a.s.l. (Zoldo Alto, Belluno, Veneto), 33T TM7642, I. Niero leg. 12/09/2015 (1 sp, INC).  
 - La Muda, path 546 to Forcella Moschesin, 715m a.s.l. (Agordo, Belluno, Veneto), 33T TM7726, I. Niero leg. 28/06/2002 (1 sp, INC; Fig. 5 D).  
 - E slope of Sant’Anna Lake, Comelico Valley, 1400m a.s.l. (Comelico Superiore, Belluno, Veneto), 33T UM0761, I. Niero leg. 09/07/2003 (1 sp, INC).  
 - Path 137 on the left of Oregone Stream, Visdende Valley, 1505m a.s.l. (San Pietro di Cadore, Belluno, Veneto), 33T UM2366, I. Niero leg. 16/09/2000 (1 sp, INC).  
 - Costa Campo di Sopra, Pizzoc Mountain, Cansiglio Natural Park, 1320m a.s.l. (Fregona,

Treviso, Veneto), 33T TM9702, I. Niero leg. 23/06/2001 (1 sp, INC).

- Prescudin, Barcis, 550m a.s.l. (Barcis, Pordenone, Friuli-Venezia Giulia), 33T UM0618, M.M. Giovannelli leg. 14/05/1990 (1 sp, MFSN; Fig. 6 A-B).

- Paleontological pad, Preone, 1545m a.s.l. (Preone, Udine, Friuli-Venezia Giulia), 33T UM3539, S. Cianfanelli, M. Calcagno, G. & L. Manganelli leg. 27/08/1999 (1 sp, MZUF GC/52618).

- Pad CAI n°401, Passo Monte Croce Carnico, 1440m a.s.l. (Paluzza, Udine, Friuli-Venezia Giulia), 33T UM4263, S. Cianfanelli & M. Calcagno leg. 29/08/1999 (1 sp, MZUF GC/9018).

- Planda Purcei, Monte San Simeone, 1200m a.s.l. (Bordano, Udine, Friuli-Venezia Giulia), 33T UM5433, S. Cianfanelli & M. Calcagno leg. 22/08/2005 (3 sp, MZUF GC/21130; Figs 1 J-L, 6 D-E).

- Monte Strabut, slope NE, 700m a.s.l. (Tolmezzo, Udine, Friuli-Venezia Giulia), 33T UM4942, G. Nardi, A. Braccia, R. Frassine & I. Niero leg. 04/09/2010 (1 sp, GNC).

- Near Staboli Prà di Lovenza, Illeggio, Val di Lunze, 800m a.s.l. (Tolmezzo, Udine, Friuli-Venezia Giulia), 33T UM5447, S. Cianfanelli & M. Calcagno leg. 05/08/1999 (1 sp, MZUF GC/52619, stub MB/102; Fig. 6 C).

- Sella Nevea, 1200m a.s.l. (Chiusaforte, Udine, Friuli-Venezia Giulia), 33T UM8338, G. Nardi & A. Braccia leg. 29/05/2003 (1 sp, GNC).

- Gran Monte, slope N, Val Torre, 800m a.s.l. (Lusevera, Udine, Friuli-Venezia Giulia), 33T UM7029, M.M. Giovannelli leg. 08/06/1996 (1 sp, MFSN).

- Malga Saisera, Saisera Valley, 1100m a.s.l. (Malborghetto-Valbruna, Udine, Friuli-Venezia Giulia), 33T UM8246, I. Niero leg. 28/06/1997 (1 sp, INC).

- N slope of Colle di Medea, 80m a.s.l. (Medea, Gorizia, Friuli-Venezia Giulia), 33T UL7786, I. Niero leg. 23/02/2008 (1 sp, INC).

- Botazzo, 200m a.s.l. (San Dorligo della Valle, Trieste, Friuli-Venezia Giulia), 33T VL1252, M. Bodon leg. 22/09/1983 (1 sp, FGC; Fig. 6 F-G).