

On the infrageneric systematics of the genus *Arion* FÉRUSSAC, 1819 (Mollusca, Pulmonata)

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Summary

This paper deals with the infrageneric systematics of the genus *Arion*. It presents a historical account, after which the infrageneric position of *A. intermedius* is discussed. This species is included in the subgenus *Kobeltia*, of which the type species is reconsidered. Next, a discussion on the infrageneric allocation of *A. lusitanicus* and *A. flagellus* is provided. Both species are provisionally placed in the subgenus *Mesarion*, which in turn may be grouped with *Arion* s.s. in the "chromosomal group" *Lochea* ($n \leq 27$). *Kobeltia* and *Carinarion* may then constitute the "chromosomal group" *Prolepis* ($n \geq 27$). This classification relies on chromosomal, electrophoretic, genital and morphological characters. Yet, the currently available data do not allow a reliable phylogenetic analysis, so that no taxonomic ranks are attributed to the retained groupings. Nevertheless, some tentative phylogenetic and evolutive hypotheses are presented. Thereby special attention is paid to 1) chromosome numbers, 2) electrophoretic data, 3) breeding systems, 4) spermatophores and 5) the complexity of genitalia and mating behaviour.

Key-words: *Arion*, breeding systems, chromosomes, electrophoresis, genitalia, spermatophores, systematics, type species.

Résumé

Cette contribution est consacrée à la systématique infragénérique du genre *Arion*. Un aperçu historique du sujet est présenté, après quoi la position infragénérique d'*A. intermedius* est discutée. Cette espèce est classée dans le sous-genre *Kobeltia*, dont l'espèce-type est commentée. La discussion se poursuit par l'attribution infragénérique d'*A. lusitanicus* et d'*A. flagellus*. Provisoirement, on propose de placer les deux espèces dans le sous-genre *Mesarion*. Puis, on présente une tentative de classification du genre *Arion* en retenant deux grandes unités chromosomiques (*Lochea* et *Prolepis*). Chacune d'elles est composée de deux groupes d'espèces (*Arion* s.s. et *Mesarion* pour *Lochea*, *Kobeltia* et *Carinarion* pour *Prolepis*). Cette classification est fondée sur des caractères chromosomiques, électrophorétiques, génitaux et morphologiques. Cependant, la connaissance actuelle de la systématique du genre *Arion* n'étant pas suffisante pour permettre une analyse phylogénétique, cela ne permet pas de définir le niveau taxonomique des groupements distingués. Néanmoins, on propose quelques hypothèses phylogénétiques et évolutives concernant 1) les nombres chromosomiques, 2) les données électrophorétiques, 3) les systèmes reproducteur, 4) les spermatophores et 5) la complexité de l'appareil génital.

Mots-clés: *Arion*, systèmes reproducteur, chromosomes, électrophorèse, appareil génital, spermatophores, systématique, espèce-type.

General introduction

The systematics of the terrestrial slugs of the family Arionidae in general, and those of the genus *Arion* FÉRUSSAC,

1819 in particular, have always been controversial. No one so far has been able to present a convenient and generally accepted generic and infrageneric classification or phylogeny of these slugs. We propose and discuss here some reflections and hypotheses on this subject. For the authorship of the arionid genus- and speciesgroup names used in the text, we refer to appendix 1.

Historical account

Before LINNAEUS (1758), slugs were usually referred to as "*Limax*" or "*Cochleis nudis*" (ÖKLAND, 1922). The former of these names was subsequently selected by LINNAEUS (1758) to denote five terrestrial slugs, including *Limax ater* and *L. rufus*. Later, LINNAEUS (1761, 1767) extended his "genus" *Limax* to include some marine species too.

FÉRUSSAC (1819) divided *Limax* into several "genera", one of which was referred to as *Arion*. It contained the species with the following characters (original language): "Cuirasse partielle ou nulle; tentacules rétractiles. Tétracères. Cuirassées antérieurement. Cavité pulmonaire antérieure; orifice au bord droit de la cuirasse, antérieurement. Orifice du rectum, près celui de la respiration. Un pore muqueux terminal. Organes de la génération réunis: Orifice sous celui de la respiration. Point de lèvres ou tentacules buccaux: des papilles; des mâchoires. Une couche de poussière calcaire et graveleuse, intérieurement dans la cuirasse." (FÉRUSSAC, 1821). This genus appeared to be so peculiar, that GRAY (in TURTON, 1840) created a separate family for it, viz. the Arionidae.

Several authors attempted to split the genus *Arion* into smaller taxa. MOQUIN-TANDON (1855) distinguished two "subgenera", namely *Lochea* for the species with a shellplate consisting of loose, discrete granulae, and *Prolepis* for the species having a shellplate consisting of an irregular, but coherent aggregate of granulae. The former group contained "*A. subfuscus*" and "*A. rufus*" (i.c. *A. lusitanicus*), while the latter group contained "*A. hortensis*".

MABILLE (1868) constituted the genus *Baudonia* to include *B. timida* and *B. montana*. These species would differ from *Arion* by 1) the anteriorly broadened and depressed body, 2) the clearly delimited head, 3) the smooth mantle and

4) the short tentacles. HEYNEMANN (1870) also used *Baudonia*, although he considered it, together with *Lochea* and *Prolepis*, as a subgenus of *Arion*. Most authors, however, rejected *Baudonia*, because they argued that the characters mentioned by MABILLE (1868) were much too variable to define higher taxa (COLLINGE, 1897).

MABILLE (1870) described still another arionid genus, viz. *Carinella*, for which *A. bourguignati* (= *A. fasciatus* s.l. see COCKERELL, 1891a,b; COLLINGE, 1892a,b, 1893) acted as type species. *Carinella* included the species with a "dorsal keel", but this is usually an optical effect caused by the mediodorsal tubercle row. The new genus was therefore rejected by COLLINGE (1897). Later it was also shown that even if *Carinella* was accepted as a taxon, the name *Carinella* itself was not available because of the homonymy with *Carinella* JOHNSTON, 1833 (Nematomorpha) and *Carinella* SOWERBY, 1839 (Mollusca; error pro Cancinelle ADANSON, 1757) (SHERBORN, 1924).

SEIBERT (1873) created the genus *Kobeltia* for a species he referred to as *A. hortensis*. Unfortunately, SEIBERT (1873) did not support his opinion with arguments. So, subsequent authors refrained from using the name *Kobeltia*, except for mentioning it in synonymy lists (e.g. LESSONA, 1881; LESSONA & POLLONERA, 1882; COCKERELL, 1892; TAYLOR, 1907), until HESSE (1926) employed it to denote a "section" in the genus *Arion*. Nowadays, the name *Kobeltia* is commonly used throughout malacological literature.

LESSONA (1881) and LESSONA & POLLONERA (1882) initially adopted the two subgenera of MOQUIN-TANDON (1855) (*Lochea* and *Prolepis*). In this system, *A. bourguignati* was assigned to *Prolepis*, but later POLLONERA (1885) transferred *A. bourguignati* and *A. subcarinatus* to MABILLE'S (1870) *Carinella*, which received a rank comparable to that of *Lochea* and *Prolepis*.

SIMROTH (1885a) refuted MOQUIN-TANDON'S (1855) arrangement by arguing that the degree of aggregation of the granulae under the mantle is an unreliable character. Alternatively, SIMROTH (1885a) divided the genus by using the structure of the genital atrium and so he distinguished the Monoatriidae and the Diatriidae. In the former group, the genital atrium would consist of a single vestibule, whereas in the latter it would be composed of two vestibules: an upper (distal) one comprising the terminations of the genital ducts and a lower (proximal) one containing the genital porus. In this scheme, SIMROTH (1885a) assigned *A. minimus* (= *A. intermedius*), *A. subfuscus* and *A. brunneus* to the Monoatriidae and *A. empiricorum*, *A. hortensis* and *A. timidus* to the Diatriidae, but he also admitted that *A. bourguignati* did not fit well in this system. So this species received a separate, though undefined, position.

According to SIMROTH (1885a) the division in Mono- and Diatriidae was also supported by the colour patterns. The Monoatriidae would have dark lateral bands with both margins sharply delimited, whereas in the Diatriidae, only the upper margin would be clearly defined.

SIMROTH'S (1885a) classification, was vigorously rejected by POLLONERA (1887) who wrote: "Questa divisione è, a mio parere, completamente illusoria, ...". He argued that

the genital atrium in arionids is too variable to be used for systematics above the species level. This could not be refuted and hence SIMROTH (1887, 1891) himself, abandoned his classification.

POLLONERA (1887) distinguished four species-groups. These groups were classified in two larger units, which were related to MOQUIN-TANDON'S (1855) *Lochea* and *Prolepis* (fig. 1). However, in later publications, POLLONERA (1889, 1890), avoided to link his species-groups with these "subgenera" (appendix 2).

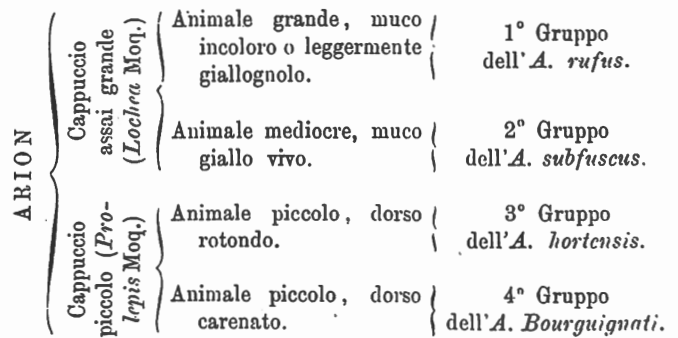


Fig. 1. - Infrageneric systematics of the genus *Arion* as proposed by POLLONERA (1887).

This system was adopted by COLLINGE (1892a), who shortly afterwards, splitted POLLONERA'S *A. hortensis* group into two separate units: one including *A. hortensis*, the other containing *A. intermedius* (COLLINGE, 1897). Hence, COLLINGE (1897) proposed a classification involving five infrageneric species-groups (appendix 3).

This arrangement was formalized by HESSE (1926), who distinguished three subgenera in *Arion*, viz. *Letourneuxia*, *Ariunculus* and *Arion* s.s. This latter comprised five "sections" corresponding to COLLINGE'S (1897) species-groups. HESSE (1926) attached names to these "sections", selected type species for them and provided diagnoses (appendix 4). TORRES MINGUEZ (1925) did not accept COLLINGE'S (1897) ideas, but relied on POLLONERA'S work (1887, 1889, 1890) to define three infrageneric units viz. the *Majorium* group (= *A. empiricorum* group), the *Mediocrum* group (= *A. subfuscus* group) and the *Parvorum* group (= *A. hortensis* + *A. bourguignati* groups). This latter group was further divided in a *subcylindricus* section for the species with a rounded back (e.g. *A. hortensis*) and a *pseudocarinatus* section for the species with a "keeled" back (e.g. *A. bourguignati*).

Most subsequent authors, however, followed COLLINGE'S (1897) and thus also HESSE'S (1926) classification (e.g. MERMOD, 1930; EHRMANN, 1933) or modified it with respect to three items: 1) the subgenera *Letourneuxia* and *Ariunculus* were removed from *Arion* and received a generic rank, 2) HESSE'S (1926) sections were raised to subgenera and 3) the name *Lochea* was replaced by *Arion* (GERMAIN, 1930), while the type species of this subgenus (*A. empiricorum*) was specified to be *A. ater* (ICZN Op. 335).

Despite the common use of HESSE'S classification, the infrageneric systematics of *Arion* have remained contro-

versial (PILSBRY, 1948; QUICK, 1960; BOATO *et al.*, 1983; GITTENBERGER *et al.*, 1984; MARTÍN & GOMEZ, 1988). FORCART (1965) even suggested that the differences between *A. ater* and *A. hortensis* do not warrant a subgeneric distinction.

In this context, DAVIES (1987) formulated an alternative scheme based on the form of the spermatophores and the structure of the eggs. DAVIES (1987) distinguished three groups:

(1) *Mesarion* + *Arion* s.s.: eggs robust, chalky, often large. Spermatophores elongate, tapering, with a single longitudinal ridge (Pl. 3-5).

(2) *Kobeltia* + *Microarion*: eggs thinly coated, soft and sticky, small. Spermatophores truncated, more or less hooked posteriorly (fig. 3).

(3) *Carinarion*: eggs with a tough flexible integument. Spermatophores elongate, polygonal, with fine longitudinal ridges (Pl. 1-2).

This classification will be dealt with further on, where we will also propose our own current view on the infrageneric systematics of *Arion*.

The infrageneric position of *A. intermedius*

Introduction

The allocation of *A. intermedius* to its own subgenus (*Microarion*) has been debated by several authors who suggested that the species should not be separated from the *A. hortensis* group (*Kobeltia*) (WALDÉN, 1976, 1983; KOFER, 1977; DAVIES, 1979; BACKELJAU, 1985; BACKELJAU & DE WINTER, 1987; MANGANELLI & GIUSTI, 1988). It appears as if there are indeed only very few characters by which *Microarion* really differs from *Kobeltia* (see diagnoses in appendix 3-4). In fact, only three characters can be retained: 1) the short and undifferentiated oviduct of *Microarion* vs. the long and differentiated oviduct of *Kobeltia*, 2) the absence of spermatophores in *Microarion* vs. the production of spermatophores in *Kobeltia* and 3) the absence of a ligula-like structure in *Microarion* vs. the presence of such an organ in most *Kobeltia* spp.

Before evaluating these "diagnostic" features, we will show that other features mentioned in the diagnoses of *Microarion* and *Kobeltia*, do not permit a separation of both taxa. The following comparison is based on an extensive literature survey (see references) and personal observations.

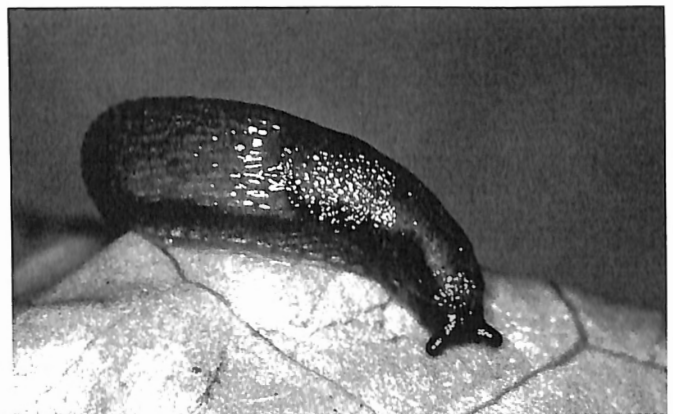
External morphology

Both *Kobeltia* spp. and *A. intermedius* have a yellowish sole and produce a yellow mucus. Furthermore, *A. intermedius* and *Kobeltia* spp. usually have dark lateral bands on the mantle and body sides (fig. 2). Generally, the colour of *A. intermedius* ranges from yellowish over brown to metal gray, whereas *Kobeltia* spp. vary from brownish over gray to deep metal blue-black, with sometimes whitish body sides. So the colour in the two taxa is highly

variable and overlapping. Moreover, colour in *Arion* spp. depends on factors such as age, food and (micro)environmental conditions (SIMROTH, 1884, 1885a; LOENS, 1890; GAIN, 1892; COLLINGE, 1897; KÜNDEL, 1916; MARTENBACH, 1940; ALBONICO, 1948; BOETTGER, 1949; ANT, 1957; CHEVALLIER, 1972; BACKELJAU, 1989a). Even the for *A. intermedius* typical warty tubercles, are not restricted to this species, since *A. owenii* may display a comparable tubercle type (DAVIES, 1977; BACKELJAU & VAN BEECK, 1986; MANGANELLI & GIUSTI, 1988). Finally, the size of slugs is such a variable character that it should only be used in a relative meaning. In this sense both *Kobeltia* spp. and *A. intermedius* are "small" species in comparison with, for example, *A. ater* s.l. and *A. subfuscus* s.l. In conclusion, the external morphology of *A. intermedius* does not warrant the separation of this species from *Kobeltia*.

Genital structures

The typical *Kobeltia* spermatophore is smooth as in *A. distinctus* (figs. 3A, 4G, but see also fig. 21) (HESSE, 1926; GERMAIN, 1930; WIKTOR, 1973; GROSSU, 1983). *A. hortensis* s.s. and *A. owenii*, however, produce spermatophores



A



B

Fig. 2. – Habitus of two *Arion* species. – A: *A. distinctus* (Wilrijk-Antwerpen, Belgium; real size ± 25 mm) – B: *A. intermedius* (Flores, Azores; real size ± 20 mm).

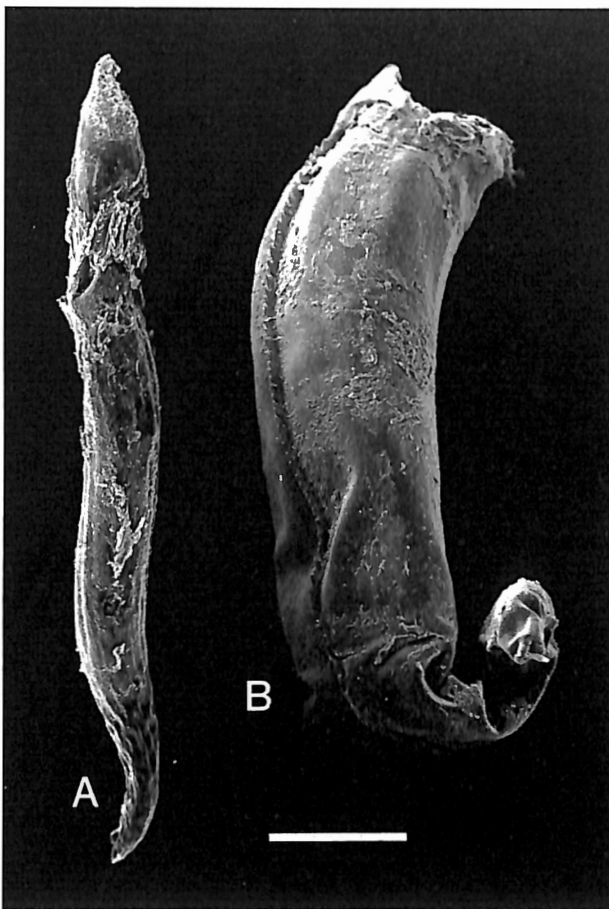


Fig. 3. – SEM photographs of two *Kobeltia* spermatophores. Scale : 1 mm. - A : *A. distinctus* (Wilrijk-Antwerpen, Belgium) - B : *A. hortensis* s.s. (Mortsel, Belgium).

which are strongly serrated (figs. 3B, 4B, 4D) (DAVIES, 1977, 1979; DE WINTER, 1984; BACKELJAU & MARQUET, 1985; BACKELJAU & VAN BEECK, 1986). So, in view of this interspecific variability among *Kobeltia* spp., we do not consider the lack of a spermatophore (*A. intermedius*) as a more fundamental difference, than the production of spermatophores which do not correspond to the diagnosis of the subgenus. Similarly, ROBERTSON (1989) remarked that “the mere presence or absence of spermatophores cannot be used to resolve relationships or to revise the higher classification of (non-stylommatophoran) gastropods”.

The same applies to the absence of a ligula and a proximal (eversible) oviducal portion in *A. intermedius* (fig. 4H). These structures lack in *A. distinctus* with a bipartite oviduct (fig. 4F), but are present in *A. distinctus* with a tripartite oviduct (fig. 4E). This polymorphism even occurs among slugs raised from the same egg clusters (DAVIES, 1977). Obviously, the fact that *A. intermedius* lacks organs, which in *Kobeltia* reveal such an intraspecific presence/absence polymorphism, cannot be relied on to support the subgeneric separation of this species.

Breeding systems

The genital differences between *A. intermedius* and *A.*

hortensis s.l. may be related to the respective breeding systems of these species, viz. probably obligate uniparental reproduction in *A. intermedius* vs. mainly outcrossing (allogamy) in *A. hortensis* s.l.

The uniparental breeding system of *A. intermedius* has been inferred from electrophoretic surveys, which showed that although *A. intermedius* is genetically polymorphic at several enzyme loci, there is no sign of heterozygosity (McCRACKEN & SELANDER, 1980; FOLTZ *et al.*, 1982, 1984; SELANDER & OCHMAN, 1983; BACKELJAU, 1985, 1989a). Thus, the species consists of a series of monomorphic genetic strains (agamic complex). Breeding experiments demonstrated that specimens reared in isolation still produce a viable offspring (CHICHESTER & GETZ, 1973; DAVIES, 1977). In addition, the courtship of *A. intermedius* has never been observed, while the spermatophore of this species is unknown. This latter observation may be correlated with the former, since spermatophores are only produced during coition (KÜNKEL, 1916; IKEDA, 1937; RUNHAM & HUNTER, 1970; LIND, 1973; WIKTOR, 1987; SHELLEY & CHASE, 1988).

The simple oviduct and the absence of a ligula in *A. intermedius*, are also characters which may be related to the abolition of courtship in this species, for these organs play an active role during copulation.

Remark that SELANDER & OCHMAN (1983) ascertained that selfing mollusc species have retained mating behaviour and the structures needed for copulation. Yet, the species to which SELANDER & OCHMAN (1983) referred, are, except for *A. intermedius*, facultative selfers, so that the preservation of functional copulatory organs and mating behaviour (including the production of spermatophores) could be expected in these cases.

In contrast to *A. intermedius*, electrophoretic analyses have shown that *A. hortensis* s.l. reproduces predominantly by allogamy (McCRACKEN & SELANDER, 1980; FOLTZ *et al.*, 1982, 1984; BACKELJAU, 1985, 1989a). The copulation and spermatophores (figs. 3-4) of these slugs are also well-known (TAYLOR, 1907; GERHARDT, 1935; QUICK, 1946, 1960; WEBB, 1950; DAVIES, 1977; BACKELJAU, 1981; DE WINTER, 1984; BACKELJAU & MARQUET, 1985; BACKELJAU & VAN BEECK, 1986). Hence, one may expect that in *A. hortensis* s.l. the copulatory organs are well-developed too. Although the genital differences between *A. intermedius* and *A. hortensis* s.l. appear to be fundamental, they only reflect alternative breeding systems. However, different reproductive modes are not reliable to distinguish taxa above the species level, for several pulmonate species have mixed breeding systems. In *A. ater* s.l. and *A. subfuscus* s.l., for example, both uniparental reproduction and outcrossing occur (WOTTON, 1893; ADAMS, 1910; KÜNKEL 1916; PERROT, 1939; GERHARDT, 1940; McCRACKEN & SELANDER, 1980; FOLTZ *et al.*, 1982, 1984; SELANDER & OCHMAN, 1983). WILLIAMSON (1959) even reported that in single egg clutches of *A. ater* s.l. always some eggs are fertilized by autosperm, whereas the remaining ones are fertilized by allosperm. A similar phenomenon was observed in the phylomicid slug *Meghimatium bilineatum* (IKEDA, 1937) and the bulimulid snail *Liguus fasciatus*

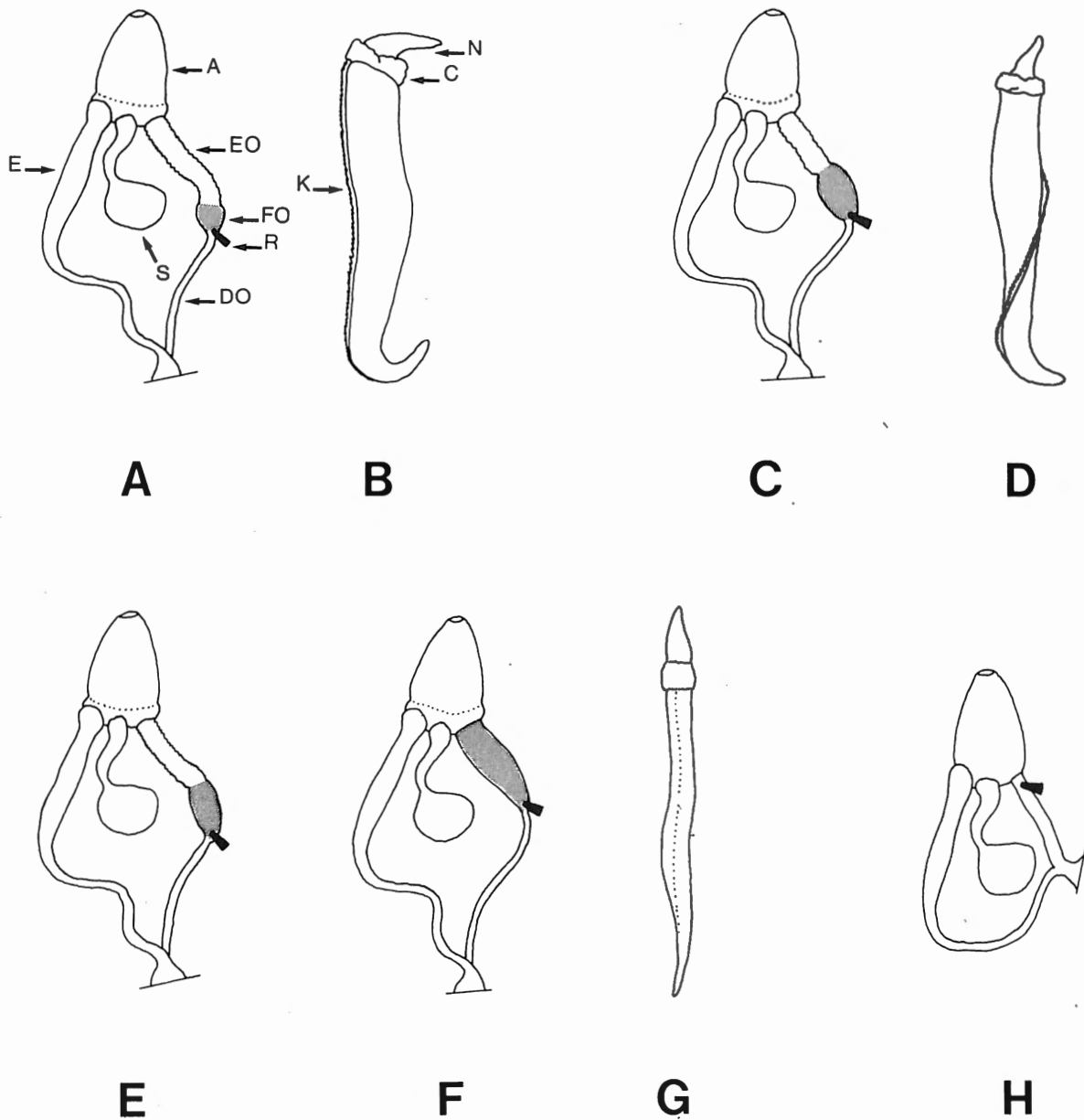


Fig. 4. - Simplified outlines of the proximal genital parts and spermatophores of four *Kobeltia* species. Abbreviations used: A = atrium; C = attachment collar; DO = distal channel of the oviduct; E = epiphallus + vas deferens; EO = eversible (proximal) portion of the oviduct; FO = firm portion of the oviduct (shaded area); K = serrated keel; N = apical nozzle; R = oviducal retractor; S = spermatheca. - A-B: *A. hortensis* s.s. (A: genitalia; B: spermatophore) - C-D: *A. owenii* (C: genitalia; D: spermatophore) - E-G: *A. distinctus* (E: genitalia with a tripartite oviduct; F: genitalia with a bipartite oviduct; G: spermatophore) - H: *A. intermedius* (genitalia).

(HILLIS *et al.*, 1987; HILLIS, 1989). There is also evidence indicating that *A. fasciatus* s.l. and *A. owenii* have mixed breeding systems too (BACKELJAU 1985, 1989a; BACKELJAU *et al.*, 1987). In *Geomalacus maculosus* uniparental reproduction was reported by OLDHAM (1942), whereas PLATTS & SPEIGHT (1988) observed the copulation of this species. Finally, there are many examples of pulmonates in which both uniparental reproduction and allogamy have been documented or suggested, e.g. *Limax flavus* and *Limax pseudoflavus* (EVANS, 1983), *Milax gagates* (QUATTRINI, 1970; FOCARDI & QUATTRINI, 1972; FOLTZ *et al.*, 1984), *Triodopsis albolabris* (MCCRACKEN & BRUSSARD,

1980), *Rumina decollata* (SELANDER & KAUFMAN, 1973; SELANDER & OCHMAN, 1983), *Partula taeniata* (MURRAY & CLARKE, 1976) and *Deroceras laeve* (MCCRACKEN & SELANDER, 1980; NICKLAS & HOFFMANN, 1981; FOLTZ *et al.*, 1982b; HOFFMANN, 1983; SELANDER & OCHMAN, 1983).

In general, still many problems need to be solved with respect to pulmonate breeding systems. For example, when uniparental reproduction is observed, it is in most cases not clear whether autogamy or parthenogenesis (automixis/apomixis) is involved. On the other hand, even if copulations, spermatophores and spermatozoa have been des-

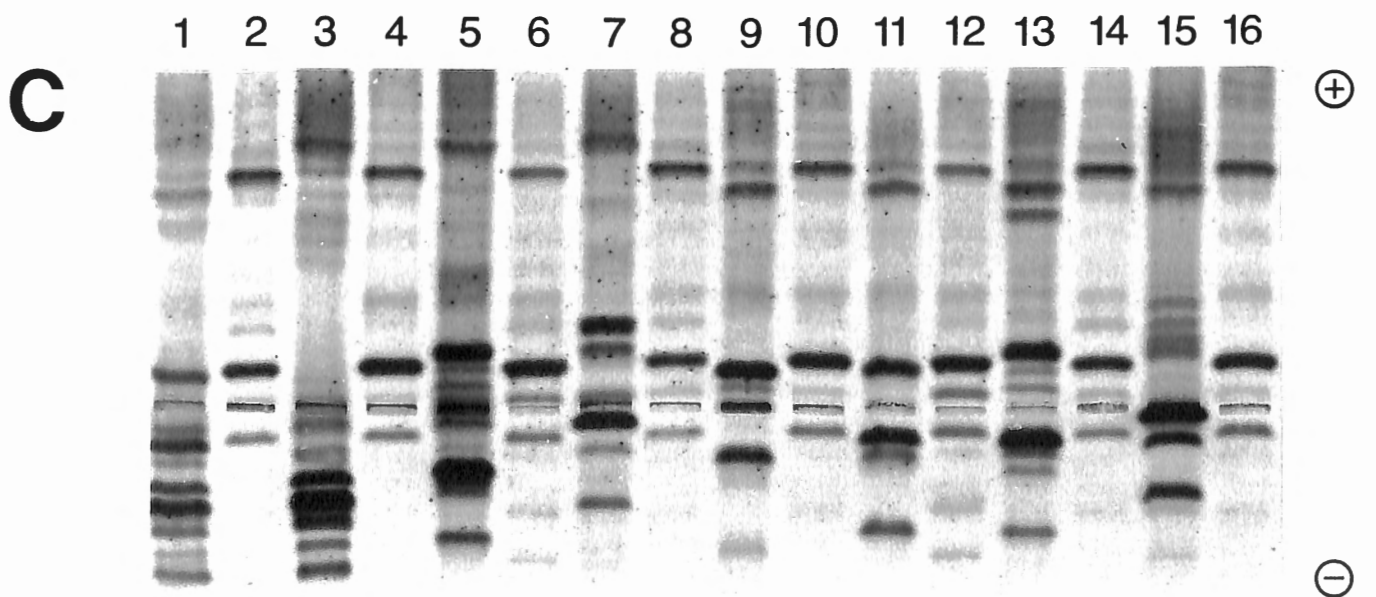
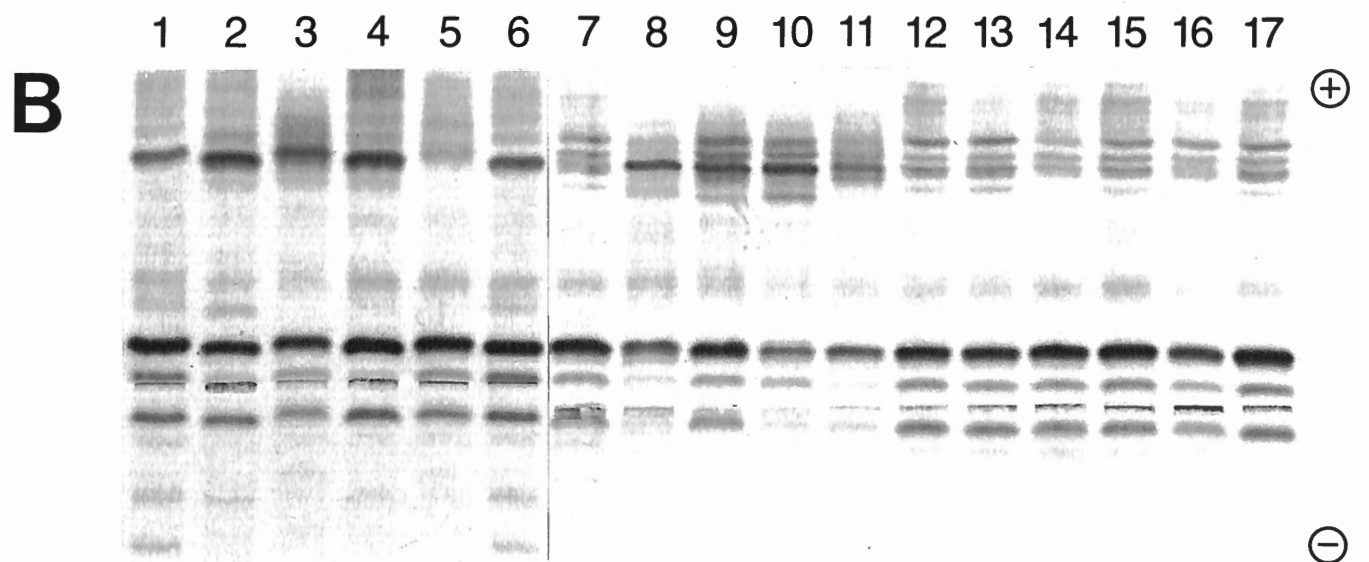
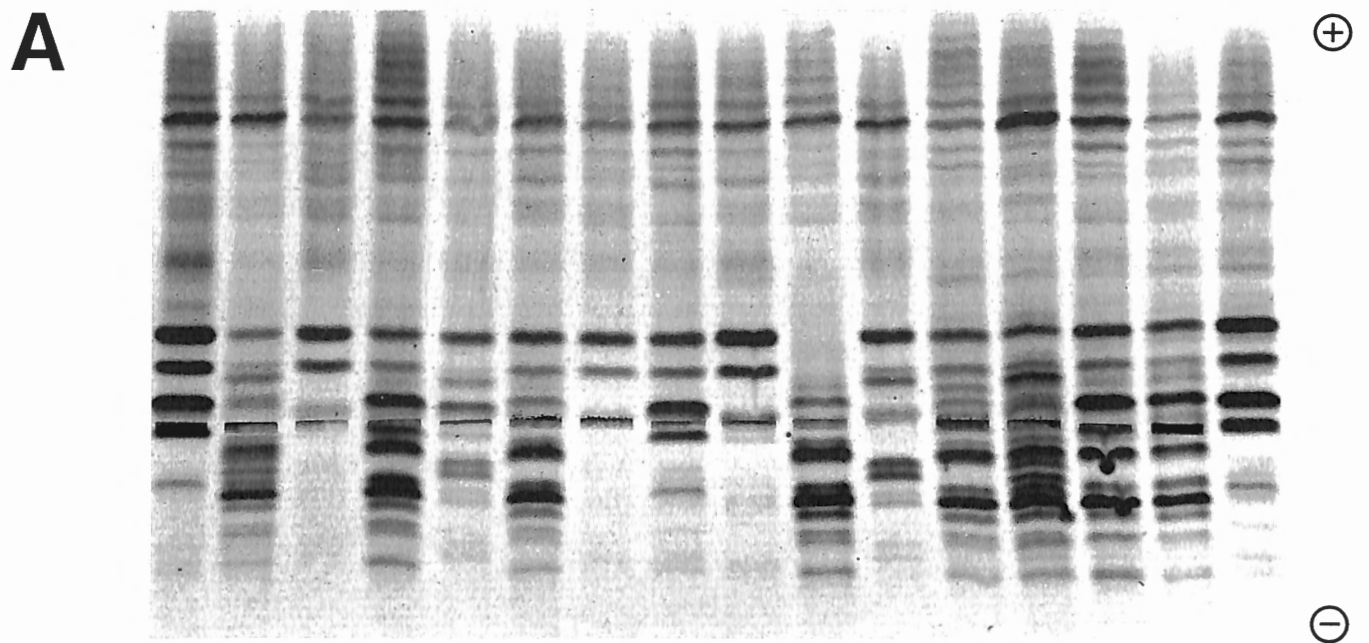


Fig. 5. – Isoelectric focusing (IEF) profiles of digestive gland esterases (EST) in four *Kobeltia* species. IEF was performed in agarose gels containing a 4-6.5 pH gradient (adapted from BACKELJAU (1985), who also provides the technical details). - A : EST variation in a single population of *A. distinctus* (Loenhout, Belgium) - B : EST variation in *A. owenii* and *A. intermedius* : 1. *A. intermedius* (Ronsele, B); 2. *A. intermedius* (Leopoldsborg, B); 3. *A. owenii* (London, GB); 4. *A. intermedius* (Boeckhout, B); 5. *A. owenii* (Buncrana, Ireland); 6. *A. intermedius* (Ronsele, B); 7. *A. owenii* (Buncrana, Ireland); 8-11. *A. owenii* (London, GB); 12-17. *A. owenii* (Buncrana, Ireland) - C : EST variation between *A. intermedius*, *A. hortensis* s.s. and *A. distinctus* : 1. *A. distinctus* (Rochefort, B); 2. *A. intermedius* (Leopoldsborg, B); 3. *A. distinctus* (Berchem-Antwerpen, B); 4. *A. intermedius* (Boeckhout, B); 5. *A. distinctus* (Wilmslow, GB); 6. *A. intermedius* (Ronsele, B); 7. *A. distinctus* (Sanderstead, GB); 8. *A. intermedius* (Leopoldsborg, B); 9. *A. hortensis* s.s. (Wellin, B); 10. *A. intermedius* (Boeckhout, B); 11. *A. hortensis* s.s. (Rochefort, B); 12. *A. intermedius* (Ronsele, B); 13. *A. hortensis* s.s. (Berchem-Antwerpen, B); 14. *A. intermedius* (Leopoldsborg, B); 15. *A. hortensis* s.s. (Wilmslow, GB); 16. *A. intermedius* (Boeckhout, B).

cribed for a species, it is not at all certain whether the eggs are also fertilized by allosperm.

In summary, the different breeding systems and the related differences in the copulatory organs of *A. intermedius* and *A. hortensis* s.l. do not support a subgeneric separation of these taxa.

Protein electrophoresis

Isoelectric focusing (IEF) of esterases in *A. hortensis* s.l. and *A. intermedius* (fig. 5), revealed a higher genic similarity (S) between *A. intermedius* and *A. owenii* (S=0.66), than between *A. owenii* and respectively *A. hortensis* s.s. and *A. distinctus* (S=0.33 and S=0.18) (fig. 6). The genic similarity between these latter two species was S=0.31 (BACKELJAU, 1985). This phenetic analysis suggests that if the three segregates of *A. hortensis* s.l. are grouped in one unit, this unit should also include *A. intermedius*.

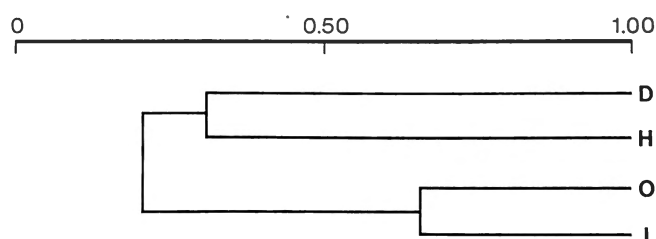


Fig. 6. – UPGMA dendrogram showing phenetic relationships between *A. hortensis* s.s. (H), *A. distinctus* (D), *A. owenii* (O) and *A. intermedius* (I), based on genic similarities obtained from IEF profiles of digestive gland esterases (fig. 5) (after BACKELJAU (1985)).

Similar results were obtained with electrophoretic analyses (PAGE) of albumen gland proteins. Especially the presence of the homologous assumed A-band in *A. hortensis* s.l., *A. intermedius* and *A. fagophilus* vs. the absence of

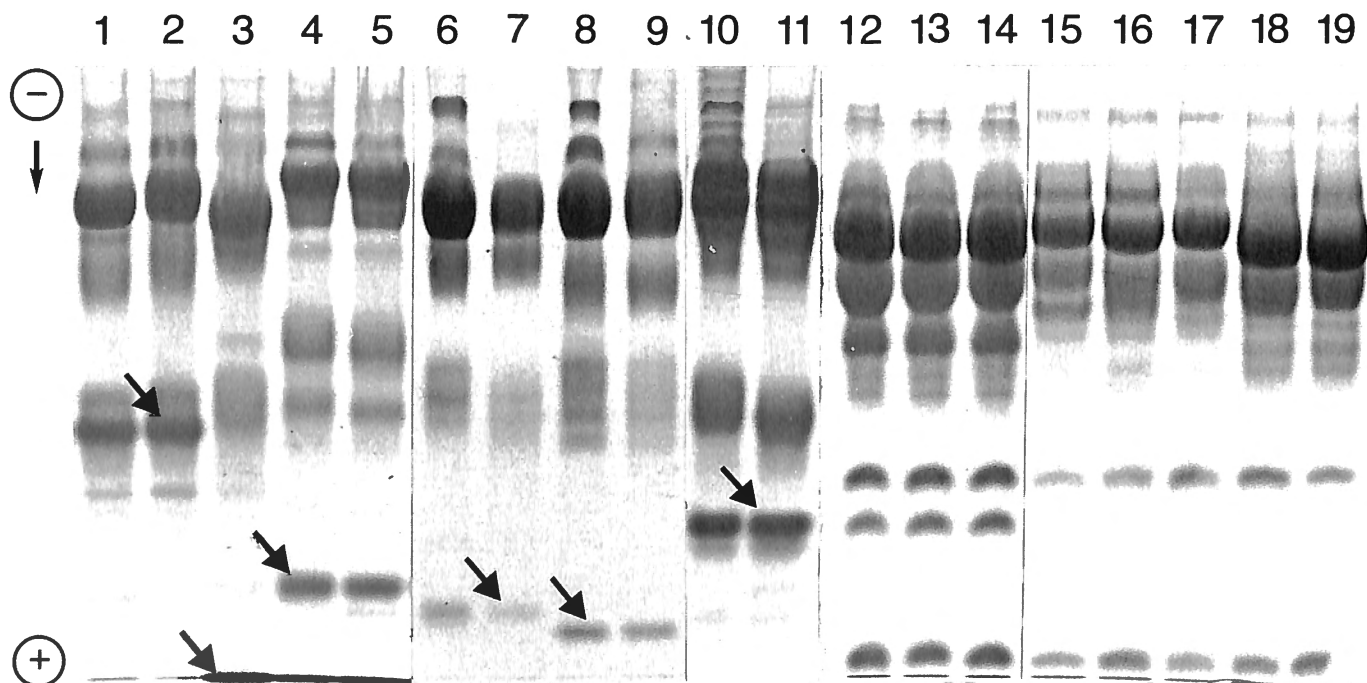


Fig. 7. – Discontinuous polyacrylamide gel electrophoresis profiles of albumen gland proteins (stained with Serva Blue G) in eight *Arion* species : *A. hortensis* s.s. (1. South Croydon, GB; 2. Mortsel, B), *A. distinctus* (3. Mortsel, B), *A. intermedius* (4-5. Mortsel, B), *A. owenii* (6-7. Buncrana, Ireland; 8-9. London, GB), *A. fagophilus* (10-11. Alsasua, E), *A. circumscriptus* (12-14. South Croydon, GB), *A. silvaticus* (15-16. Wilrijk-Antwerpen, B; 17. Standforth, GB) and *A. fasciatus* s.s. (18-19. Göteborg, S). The arrows indicate the homologous assumed A band (compiled from BACKELJAU & DE WINTER (1987) and BACKELJAU et al. (1988)).

this band in the other species studied (*A. fasciatus* s.l., *A. ater* s.l., *A. subfuscus* s.l.) (fig. 7), may indicate a higher similarity between the former three species (BACKELJAU & DE WINTER, 1987).

A. intermedius also shares more allozyme electromorphs with *A. hortensis* s.l. than with any other *Arion* species so far investigated (BACKELJAU & DE WINTER, 1987; BACKELJAU, 1989a). Recently, however, DOLAN & FLEMING (1988) contested this conclusion by referring to an allozyme study, which indicated a closer relationship between *A. intermedius* and *A. circumscriptus* (fig. 8).

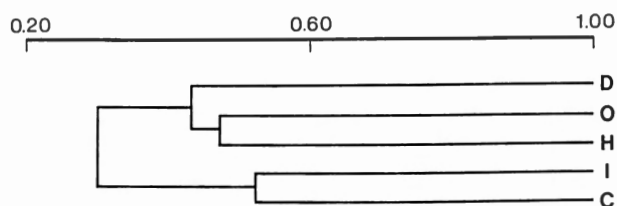


Fig. 8. - UPGMA dendrogram of Nei's genetic identities between *A. distinctus* (D), *A. hortensis* s.s. (H), *A. owenii* (O), *A. intermedius* (I) and *A. circumscriptus* (C), based on the original allele frequencies of DOLAN & FLEMING (1988) (see table 1).

This alternative viewpoint may have been provoked by DOLAN & FLEMING's (1988) technical approach, which differed from that of BACKELJAU & DE WINTER (1987) and BACKELJAU (1989a) in at least four aspects: 1) the employed electrophoretic technique (starch gel electrophoresis vs. polyacrylamide gel electrophoresis), 2) the source of tissue homogenates (body wall and foot vs. digestive gland), 3) sample preparation (in buffer vs. in aqueous sucrose solution) and 4) the electrophoretic buffers. Such methodological differences may indeed produce quite divergent results (COYNE *et al.*, 1979; RAMSHAW *et al.*, 1979; SINGH, 1979; BACKELJAU *et al.*, 1987; NEI, 1987; BACKELJAU, 1989a).

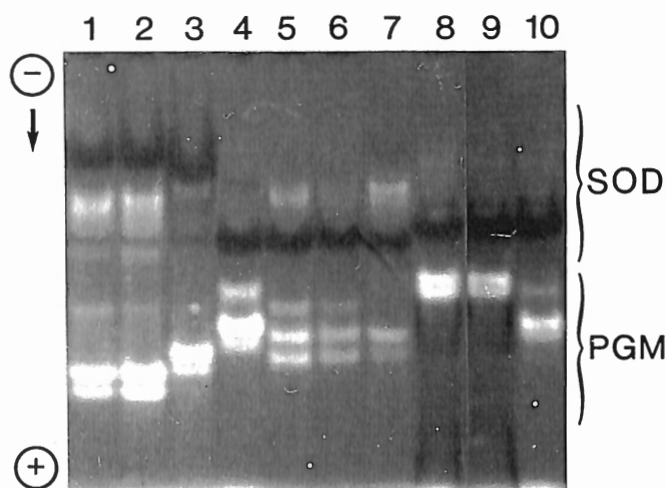


Fig. 9. - Discontinuous polyacrylamide gel electrophoresis profiles of PGM (white bands) and SOD (black bands) in digestive gland homogenates of *A. hortensis* s.s. (1-2. Wilmslow, GB), *A. fagophilus* (3. Alsasua, E), *A. subfuscus* s.l. (4. La Malou-Les-Bains, F), *A. distinctus* (5-6. Altlengbach, A), *A. intermedius* (7. Antwerpen, B), *A. fasciatus* s.s. (8-9. Göteborg, S) and *A. silvaticus* (10. Muker, GB).

A case in point is the enzyme superoxide dismutase (SOD), for which DOLAN & FLEMING (1988) reported that *A. distinctus*, *A. owenii*, *A. intermedius* and *A. circumscriptus* share the same electromorph (table 1), whereas BACKELJAU & DE WINTER (1987) found a different variant in the latter species (fig. 9; table 2). Similarly, BACKELJAU & DE WINTER (1987) demonstrated that the phosphoglucosmutase (PGM) electromorph of *A. hortensis* differs from that of *A. circumscriptus* (fig. 9; table 2), while DOLAN & FLEMING (1988) reported both species to have the same variant (table 1). On the other hand, we found one single PGM allele for *A. intermedius* and *A. distinctus* (fig. 9), whereas DOLAN & FLEMING (1988) observed two different

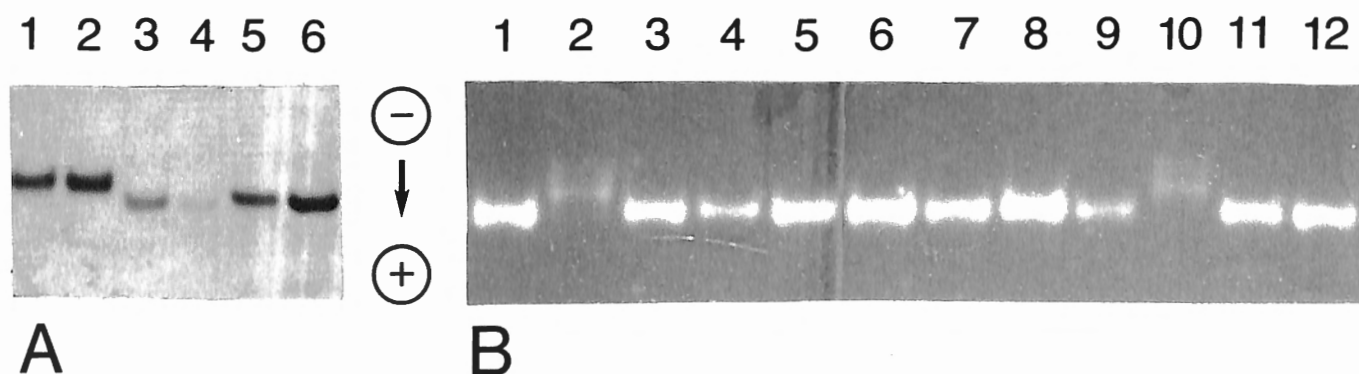


Fig. 10. - Continuous polyacrylamide gel electrophoresis profiles of IDH in digestive gland homogenates of four *Arion* species. - A: IDH polymorphism in *A. intermedius*; 1. *A. hortensis* s.s. (Antwerpen, B); 2. *A. intermedius* (Champlon, B); 3. *A. intermedius* (Alto de Lizarrusti, E); 4. *A. intermedius* (Antwerpen, B); 5. *A. silvaticus* (Antwerpen, B); 6. *A. fasciatus* s.s. (Göteborg, S). - B: IDH polymorphism in *A. hortensis* s.l.; 1-2. *A. hortensis* s.s. (London, GB); 3. *A. distinctus* (London, GB); 4. *A. hortensis* s.s. (London, GB); 5. *A. distinctus* (London, GB); 6. *A. owenii* (London, GB); 7. *A. distinctus* (Wilmslow, GB); 8. *A. hortensis* s.s. (Wilmslow, GB); 9. *A. distinctus* (Wilmslow, GB); 10-11. *A. hortensis* s.s. (Wilmslow, GB); 12. *A. distinctus* (Wilmslow, GB).

alleles (table 1). Finally, DOLAN & FLEMING (1988) resolved two isocitrate dehydrogenase loci (IDH-1 and IDH-2), which were both monomorphic within species, but polymorphic between species (table 1). BACKELJAU (1989a) found only one single, yet in *A. intermedius* polymorphic, IDH locus (IDH-p) (fig. 10A; table 2). This IDH-p locus is assumed to be identical to IDH-1 (remark from fig. 10B that IDH-p is also polymorphic in *A. hortensis* s.s.).

DOLAN & FLEMING's (1988) data are probably also influenced by the limited number of loci, populations and specimens surveyed (e.g. 14 loci in 6 specimens of *A. intermedius* and 4 specimens of *A. circumscriptus*). Small sample sizes (both among loci and numbers of specimens) can indeed bias genetic identities (NEI, 1987). In the same way they may decrease the stability of dendrograms calculated from allozyme frequency data (ARCHIE *et al.*, 1989).

Table 1

| Locus | D (N=29) | H (N=17) | O (N=25) | I (N= 6) | C (N= 4) |
|----------|----------|----------|----------|----------|----------|
| AAT-1: A | 0.621 | 1.000 | — | — | — |
| B | — | — | — | 1.000 | — |
| C | — | — | 1.000 | — | — |
| D | 0.379 | — | — | — | — |
| E | — | — | — | — | 1.000 |
| MDH-1: A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| MDH-2: A | 0.983 | — | — | 0.500 | — |
| B | 0.017 | 1.000 | 1.000 | 0.500 | 1.000 |
| MDH-3: A | 1.000 | — | 1.000 | — | — |
| B | — | 1.000 | — | 1.000 | 1.000 |
| ENO-1: A | 1.000 | — | — | — | — |
| B | — | — | 1.000 | 1.000 | — |
| C | — | 1.000 | — | — | — |
| D | — | — | — | — | 1.000 |
| PEP-1: A | 1.000 | 1.000 | — | — | 1.000 |
| B | — | — | 1.000 | 1.000 | — |
| PEP-2: A | — | — | 1.000 | 1.000 | — |
| B | 1.000 | 1.000 | — | — | 1.000 |
| PEP-3: A | 1.000 | — | — | — | — |
| B | — | — | — | 1.000 | 1.000 |
| C | — | 1.000 | 1.000 | — | — |
| IDH-1: A | 1.000 | 1.000 | 1.000 | — | — |
| B | — | — | — | 1.000 | 1.000 |
| IDH-2: A | 1.000 | 1.000 | 1.000 | — | 1.000 |
| B | — | — | — | 1.000 | — |
| MAE-1: A | 1.000 | — | — | — | — |
| B | — | 1.000 | 1.000 | — | — |
| C | — | — | — | 1.000 | 1.000 |
| SOD-1: A | 1.000 | — | 1.000 | 1.000 | 1.000 |
| B | — | 1.000 | — | — | — |
| PMI-1: A | 1.000 | 1.000 | 1.000 | — | — |
| B | — | — | — | 1.000 | 1.000 |
| PGM-1: A | 1.000 | — | — | — | — |
| B | — | — | 1.000 | — | — |
| C | — | 1.000 | — | — | 1.000 |
| D | — | — | — | 1.000 | — |

Allele frequencies for 14 enzyme loci in *A. distinctus* (D), *A. hortensis* s.s. (H), *A. owenii* (O), *A. intermedius* (I) and *A. circumscriptus* (C) according to DOLAN & FLEMING (1988). The alphabetic allele designations do not necessarily correspond to those of DOLAN & FLEMING (1988) for we did not consider *Deroceras reticulatum*.

Table 2

| Locus | D (N=29) | H (N=17) | O (N=25) | I (N= 6) | C (N= 4) |
|-----------|----------|----------|----------|----------|----------|
| IDH-p : A | 1.000 | 1.000 | 1.000 | 0.500 | – |
| B | – | – | – | 0.500 | 1.000 |
| SOD-1 : A | 1.000 | – | 1.000 | 1.000 | – |
| B | – | – | – | – | 1.000 |
| E | – | 1.000 | – | – | – |
| PGM-1 : A | 1.000 | – | – | – | – |
| B | – | – | 1.000 | – | – |
| C | – | 1.000 | – | – | – |
| D | – | – | – | 1.000 | – |
| E | – | – | – | – | 1.000 |
| AMY-1 : A | 1.000 | – | 1.000 | 1.000 | – |
| B | – | 1.000 | – | – | 1.000 |
| PGD-1 : A | 1.000 | 1.000 | 1.000 | 1.000 | – |
| B | – | – | – | – | 1.000 |

Allele frequencies of 3 enzyme loci (IDH-p, SOD-1 and PGM-1) studied by DOLAN & FLEMING (1988), but modified after BACKELJAU (1989a) and allele frequencies of 2 additional loci (AMY-1 and PGD-1) according to BACKELJAU & DE WINTER (1987). Sample sizes were deliberately kept identical to those of DOLAN & FLEMING (1988). Same abbreviations as in table 1. Remark that the PGM alleles of *A. intermedius* and *A. distinctus* are considered to be different, even though in fig. 9 they appear identical. Yet, since electrophoresis only detects differences between alleles (not similarities), we keep on using DOLAN & FLEMING's (1988) data here and change only the similarity these authors reported for the PGM alleles of *A. hortensis* s.s. and *A. circumscriptus*.

We reanalyzed DOLAN & FLEMING's (1988) work (table 1) by considering our alternative data for SOD, IDH and partly PGM (figs. 9-10; table 2) and by including two additional loci for which BACKELJAU (1987) and BACKELJAU & DE WINTER (1987) provided the necessary allele frequencies, viz. amylase (AMY) and phosphogluconate dehydrogenase (PGD) (table 2). This is justified since electrophoretic techniques only detect allozyme differences, but not allozyme similarities (FERGUSON, 1980). Therefore, we retained DOLAN & FLEMING's (1988) data for PGM in *A. intermedius* and *A. distinctus*, while we replaced their data for PGM in *A. hortensis* s.s. and *A. circumscriptus* (table 2). In addition, adding loci to a locus-by-locus analysis may increase the accuracy of the genetic identities obtained (NEI, 1987).

After modifying DOLAN & FLEMING's (1988) allele frequency matrix (tables 1-2), we considered the following 10 data combinations :

- all loci minus IDH-1 and IDH-2 (12 loci);
- all loci minus IDH-2, but plus IDH-1 (13 loci);
- all loci minus IDH-2, but plus IDH-1, AMY-1 and PGD-1 (15 loci);
- all loci minus IDH-1, IDH-2, AMY-1 and PGD-1, but plus IDH-p (13 loci);
- all loci minus IDH-1, IDH-2 and AMY-1, but plus IDH-p and PGD-1 (14 loci);
- all loci minus IDH-1, IDH-2 and PGD-1, but plus IDH-p and AMY-1 (14 loci);
- all loci minus IDH-1 and IDH-2, but plus IDH-p, AMY-1 and PGD-1 (15 loci);

- all loci minus IDH-1, IDH-2 and IDH-p, but plus AMY-1 and PGD-1 (14 loci);
- all loci minus IDH-1, AMY-1 and PGD-1, but plus IDH-2 and IDH-p (14 loci);
- all loci minus IDH-1, but plus IDH-2, IDH-p, AMY-1 and PGD-1 (16 loci)

These combinations were analyzed with the BIOSYS-1 program (release 1.7) of SWOFFORD & SELANDER (1981). For each data set, NEI's (1978) unbiased genetic identities (I) were calculated between all pairs of species. These values were used to construct UPGMA dendrograms.

In this way, we obtained four different topologies (fig. 11), of which one corresponded to that of DOLAN & FLEMING's (1988) dendrogram (fig. 8). This topology involves 2 dendrograms in fig. 11. It is the only one in which *A. intermedius* is clustered with *A. circumscriptus*, for in the three other topologies *A. intermedius* is clustered with *A. owenii* (8 out of 10 dendrograms; fig. 11)! In these 3 topologies, *A. intermedius* and *A. owenii* reveal the highest genetic identities of all species pairs investigated, ranging from $I=0.469$ to $I=0.554$. As an example, we give in table 3 NEI's (1978) unbiased genetic identities and distances between all species pairs for the combination of all modified allozyme data (see tables 1-2), excluding IDH-1, but including IDH-p, IDH-2, AMY-1 and PGD-1 (total of 16 loci).

In 5 dendrograms the *A. intermedius/A. owenii* bifurcation is clustered with a bifurcation containing *A. distinctus* and *A. hortensis* s.s. ! These four species hence form a single group, which stands in opposition to *A. circumscriptus*.

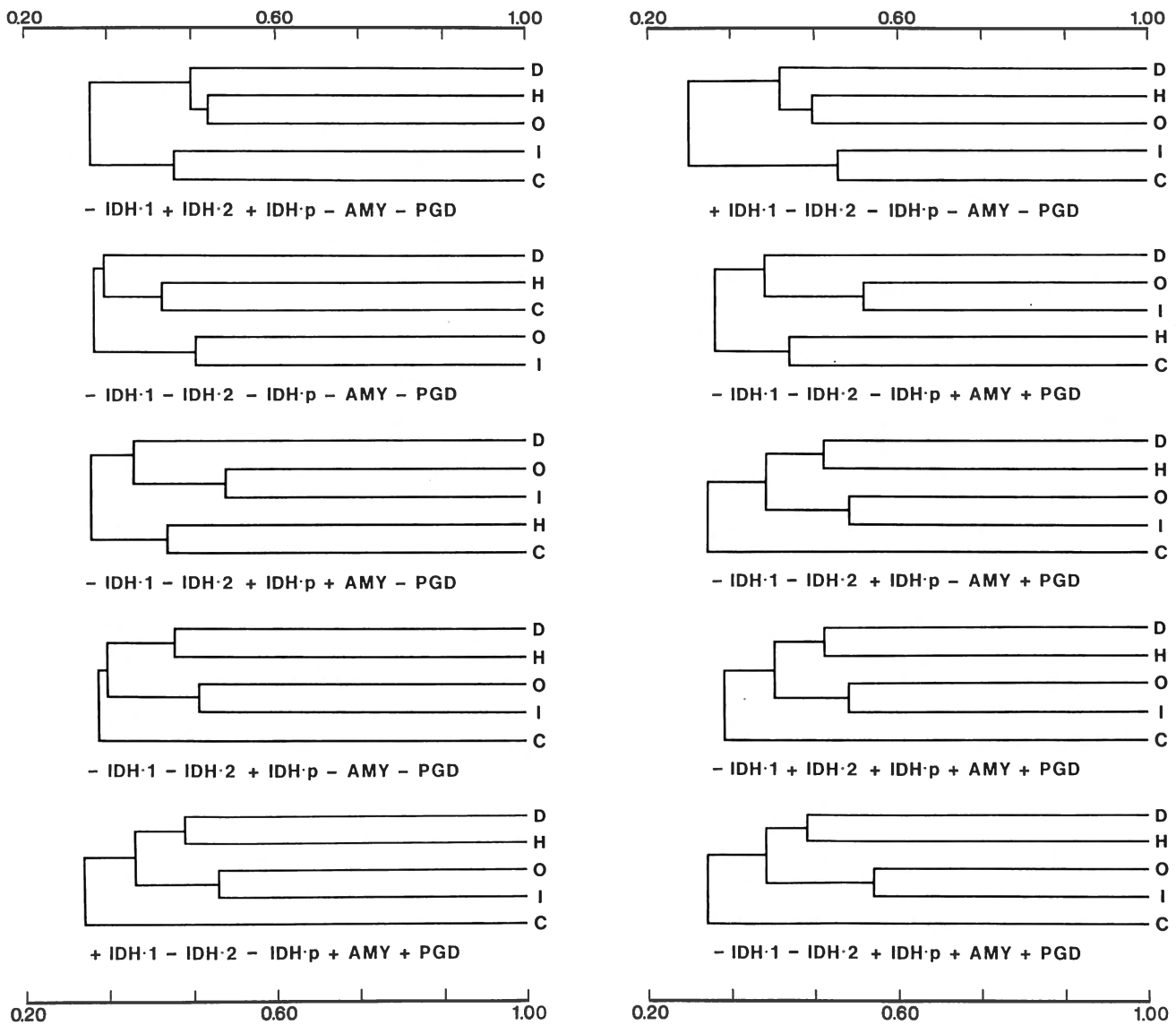


Fig. 11. – UPGMA dendrograms of Nei's unbiased genetic identities between *A. distinctus* (D), *A. hortensis* s.s. (H), *A. owenii* (O), *A. intermedius* (I) and *A. circumscriptus* (C) based on the modified allele frequencies of DOLAN & FLEMING (1988) (see table 2). The investigated locus combinations, are indicated below the dendrograms.

Table 3

| | D | H | O | I | C |
|---|-------|-------|-------|-------|-------|
| D | – | 0.485 | 0.509 | 0.329 | 0.255 |
| H | 0.723 | – | 0.500 | 0.259 | 0.438 |
| O | 0.675 | 0.693 | – | 0.518 | 0.188 |
| I | 1.112 | 1.351 | 0.658 | – | 0.388 |
| C | 1.366 | 0.827 | 1.674 | 0.946 | – |

Nei's (1978) unbiased genetic identities (above diagonal) and distances (below diagonal) between five *Arion* species, based on DOLAN & FLEMING's (1988) data (table 1), yet modified according to table 2 (excluding IDH-1, but including IDH-p, IDH-2, AMY-1 and PGD-1; hence a total of 16 loci were considered). Same abbreviations as in table 1.

This topology is identical to the topology of the dendrogram BACKELJAU (1985) constructed (fig. 6). Thus our results show that also DOLAN & FLEMING's (1988) work supports the suggestion that *A. intermedius* and *A. hortensis* s.l. belong to a single higher taxon.

Chromosome numbers

The inclusion of *A. intermedius* in *Kobeltia* is finally also confirmed by a chromosomal analysis. BEESON (1960) reported the following haploid chromosome numbers in *Arion* spp.: *A. ater* n=26, *A. rufus* n=26, *A. subfuscus* n=25, *A. circumscriptus* n=29, *A. hortensis* n=28 and *A. intermedius* n=28. Unfortunately, BEESON (1960) did not distinguish neither the segregates of *A. circumscriptus* (= *A. fasciatus* s.l.), nor those of *A. hortensis*. Yet, we do not

expect that chromosome numbers will vary among species from the same species-groups, for chromosome numbers of congeneric euthyneuran species rarely differ. Among opisthobranchs they generally even remain constant over whole orders (BURCH, 1965; BUTOT, 1967; RAINER, 1967; PATTERSON & BURCH, 1978; BOATO, 1986). Hence, karyological data can be useful to delimit euthyneuran taxa above the species rank (BURCH, 1961, 1965; BUTOT & KIAUTA, 1967, 1969; MINICHEV, 1974; PATTERSON & BURCH, 1978), even though there are a number of examples in which congeneric euthyneuran species have different chromosome numbers (BURCH, 1965; RAINER, 1967; PATTERSON & BURCH, 1978; BOATO, 1986). Yet, the chromosomal differences in many of these cases still correspond to infrageneric units (BURCH, 1965; RAINER, 1967). The genus *Arion* may represent such a situation (BACKELJAU, 1985; BACKELJAU & DE WINTER, 1987).

Conclusion

There exist no fundamental differences between *A. intermedius* and *A. hortensis* s.l. which warrant the maintenance of different higher taxa for these species. The inclusion of *A. intermedius* in *Kobeltia* is furthermore strongly supported by allozyme studies and chromosomal data. Consequently, *A. intermedius* and *A. hortensis* s.l. are for the time being united in a single species-group ("subgenus"). According to the priority rule this taxon should be referred to as *Kobeltia*, of which *Microarion* would be a subjective junior synonym.

The type species of *Kobeltia*

SEIBERT (1873) created the genus *Kobeltia* to include a species he referred to as *A. hortensis*. Recently, however, DAVIES (1977, 1979) and subsequent authors demonstrated that this name encompasses at least three different species, viz. *A. hortensis* s.s., *A. distinctus* and *A. owenii*. Thus a problem arose with respect to the actual identity of the type species of *Kobeltia*.

BACKELJAU & DE WINTER (1987) suggested this to be *A. distinctus* because SEIBERT (1873) mainly studied the fauna of Germany and referred explicitly to the work of LEHMANN (1873). This latter author specialized in the fauna of Stettin and Pommeren, two regions which nowadays belong to Poland, but which in those times were part of Germany. DE WINTER (1984) pointed out that, while *A. distinctus* is common all over West and Central Europe (including Germany and Poland), *A. hortensis* s.s., on the contrary, is a more western species, of which hitherto only one record has been reported from Germany (Gruiten, LB77, Nordrhein-Westfalen, W. Germany). KOFLER (1986) also mentioned the species from Linz in Austria, but this record needs confirmation.

A. owenii has until now only been found in the British Isles (DAVIES, 1977, 1979; BACKELJAU *et al.*, 1988). The only continental record of this species (Kiel, W. Germany,

see ZEISSLER, 1981) is based on misidentified specimens of *A. distinctus* (BACKELJAU *et al.*, 1988).

These distributional data indicate that SEIBERT's (1873) *A. hortensis* most probably involved *A. distinctus*. Moreover, subsequent descriptions of *Kobeltia* (e.g. HESSE, 1926; GERMAIN, 1930; WIKTOR, 1973) also clearly refer to this species (smooth spermatophore, position of the lateral bands).

The specification that *A. distinctus* would be the actual type species of *Kobeltia*, relied on the premise that LEHMANN (1862, 1873) and SEIBERT (1873) used the name "*A. hortensis*" to denote one of the segregates which are currently grouped in the *A. hortensis* complex. However, this appears not to be the case.

Actually, SEIBERT (1873) explicitly indicated fig. 4a of LEHMANN (1873) as type of *A. hortensis*. He also confirmed that LEHMANN's (1873) description of *A. hortensis* almost exclusively refers to what SEIBERT (1873) considered as the common and typical form of this species.

LEHMANN's (1873) description of *A. hortensis* contains the following diagnostic features (literally):

- Länge 4-5 cm. Breite 4-5 mm;
- in den Ruhe breit abgerundet;
- Schild: Länge 18 mm, Breite 5 mm;
- Grundfarbe des Thiers ist eine gelbliche oder weiss-graue, ...
- Oberhalb und Unterhalb der dunklen Binde ist die Farbe heller gelb.
- Häufig erscheint unter der dunklen Längsbinde ein intensiv citrongelber oder oranger Streif. Er fehlt aber auch zumal bei den schwärzlich grauen Stücken;
- Die Sohle ist gelblichweiss silberweiss;
- Schleim des Fusses glashell;
- Die Blase ist zungenförmig, langelliptisch."

The dimensions mentioned by LEHMANN (1873) are much too large to refer to *A. hortensis* s.l. for the mean length of the mantle in *A. hortensis* s.s. and *A. distinctus* is only about 6 mm (in alcohol, but after relaxation) (BACKELJAU & MARQUET, 1985).

Moreover, the oviduct of *A. hortensis* s.l. is almost as long as the vas deferens and epiphallus together (fig. 4), while in the genitalia of "*A. hortensis*" figured by LEHMANN (1873: Pl. VII fig. 4), the oviduct is much shorter (fig. 12). The colour of the body and the mucus as described by LEHMANN (1873) also excludes *A. hortensis* s.l., for this species produces a yellowish mucus, has an orange-yellowish sole and has a brownish to blue-blackish body without orange-yellowish bands under the dark lateral stripes (see section 3).

Finally, *A. hortensis* s.l. has a spherical, rather than a tongue-like, spermathecal bursa.

The features enumerated by LEHMANN (1873) fit remarkably well an in NW-Poland common species, which is currently referred to as *A. fasciatus*. This was already remarked by SIMROTH (1885a), who suggested that LEHMANN (1873) mixed up *A. hortensis* and *A. bourguignati* (= *A. fasciatus* s.l.). According to SIMROTH (1885a), LEHMANN's (1873) fig. 4 would be true *A. hortensis*, while his fig. 4a would refer to *A. bourguignati*. Since SEIBERT

created the genus *Kobeltia* for the species represented by this latter figure, it is obvious that he actually intended to separate *A. fasciatus*. This was confirmed by (1873) SEIBERT (1873) himself, who wrote about LEHMANN'S (1873) *A. hortensis* that "Die Beschreibungen LEHMANN'S (1873) *A. hortensis* passen auch nur auf fig. Nr. 4a". With respect to the form illustrated in LEHMANN'S (1873) fig. 4, SEIBERT (1873) stated "ich bin über diese Form noch nicht klar". Unfortunately, SEIBERT apparently never published again on this subject. Hence, it remains unknown what he planned to do with the "*A. hortensis*" with a dark body and a yellow sole.

This implies that *A. fasciatus* is the actual type species of *Kobeltia*, which in turn means that *Carinarion* (type species: *A. circumscriptus* = one of the segregates of *A. fasciatus* s.l.) would become a junior synonym of *Kobeltia*. Hence, the equivalent taxon to which *A. hortensis* s.l. belongs, would require a new name. This could be *Microarion*, for we suggested in section 3 that *A. hortensis* s.l. and *A. intermedius* belong to a single "subgenus". Yet, such a rearrangement does not serve nomenclatural stability, since after HESSE'S (1926) work, the names *Kobeltia* and *Carinarion* have always been used in their current sense. Therefore, this case has been submitted to the ICZN with the request to conserve the link between *A. hortensis* and *Kobeltia*.

The infrageneric position of *A. lusitanicus* and *A. flagellus*

The differences between these two species (which hereafter will be referred to as *A. lusitanicus* s.l.) were recently established by DAVIES (1987) and RISCH & BACKELJAU

(1989). Yet, contradictory opinions exist with respect to the infrageneric allocation of both species.

DAVIES (1987) placed *A. lusitanicus* in *Arion* s.s., while *A. flagellus* would be a *Mesarion*. RISCH & BACKELJAU (1989), however, suggested that both species are more similar to *A. subfuscus* s.l. than to *A. ater* s.l. and hence

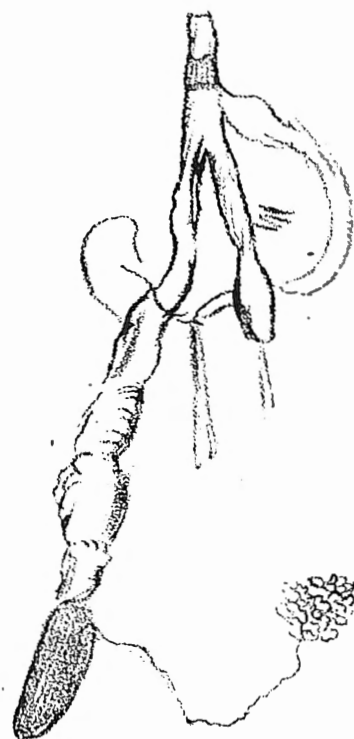


Fig. 12. - Enlarged copy of LEHMANN'S (1873) figure of the genitalia of "*A. hortensis*".

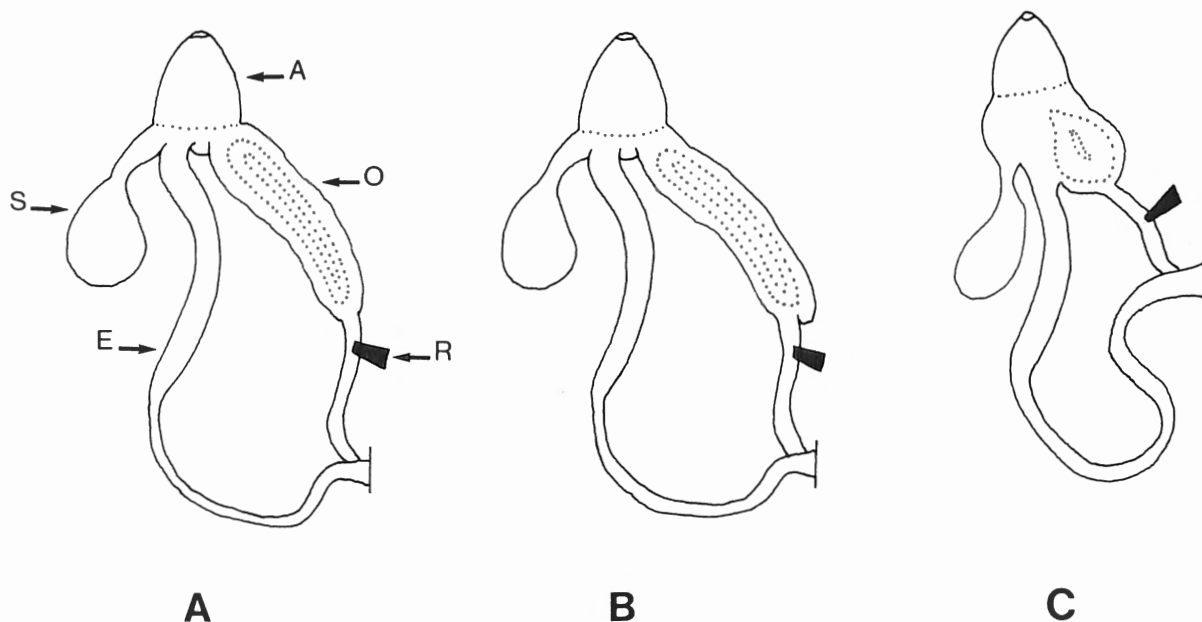


Fig. 13. - Simplified outlines of the proximal genital parts of three large *Arion* species. Abbreviations as in fig. 4, adding O = oviduct. The dotted structure is the ligula. - A : *A. lusitanicus* s.l. - B : *A. subfuscus* s.l. - C : *A. ater* s.l. - Remark : both *A. lusitanicus* s.l. and *A. subfuscus* s.l. may show a bulbous protrusion at the junction between the proximal portion of the oviduct and its distal channel (here only indicated in B).

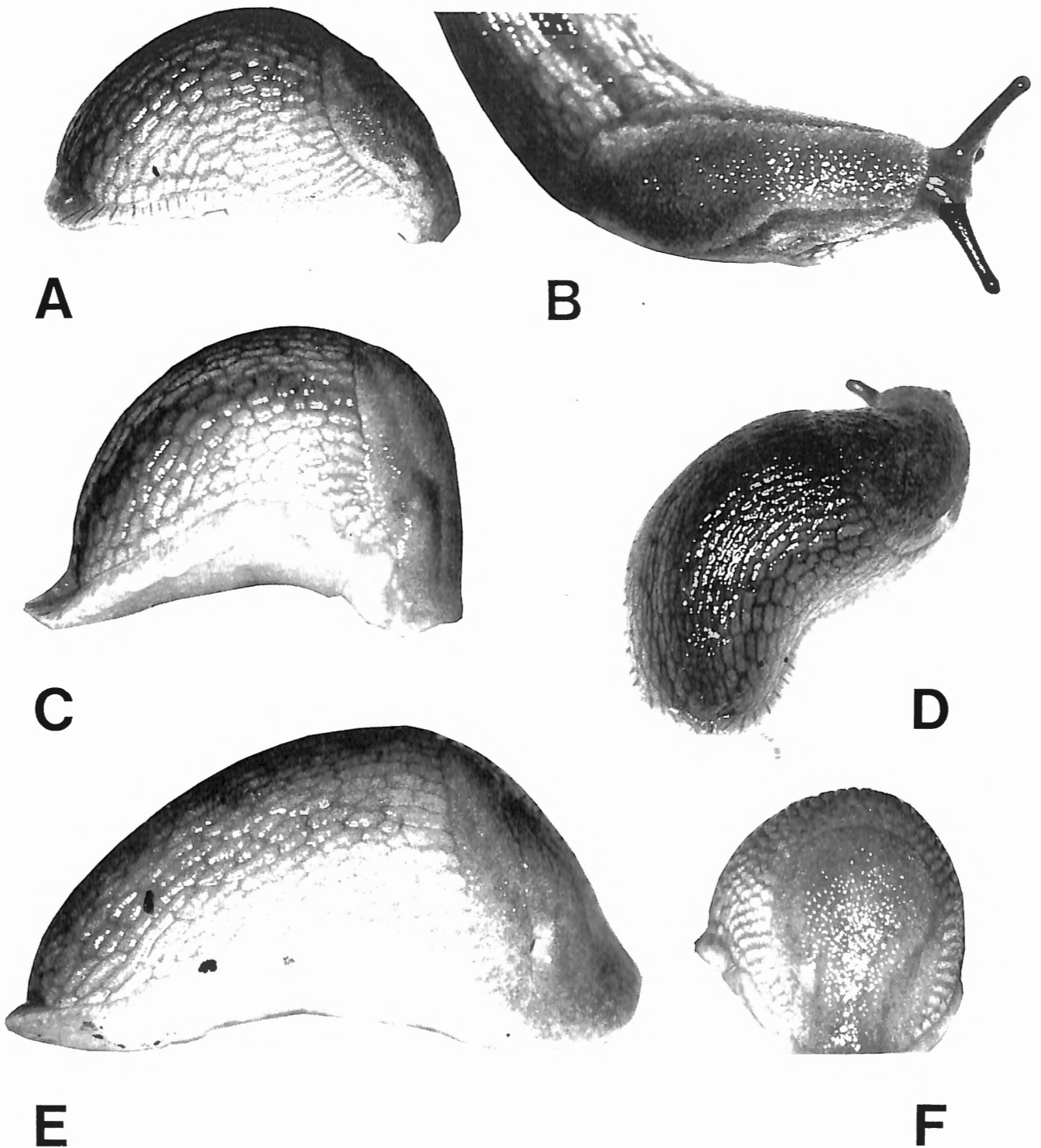


Fig. 14. – Habitus of *A. subfuscus* s.l. - A : Lago Fiorenza, I (real size ± 40 mm) - B : Tischamend, A (real size ± 45 mm) - C : Drongen, B (real size ± 40 mm) - D : same specimen as in A - E-F : Lago Fiorenza, I (real size ± 35 mm).

may be more conveniently assigned to *Mesarion*. This latter hypothesis is based on the observation that *A. lusitanicus* s.l. and *A. subfuscus* s.l. share the following features :

- Genital atrium relatively small and weakly developed; its distal compartment is almost completely absent (fig. 13A-B).
- Oviduct long and conspicuous, with a strongly developed proximal portion which contains a ligula (fig.

13A-B).

- Ligula consisting of longitudinal folds (fig. 13A-B).
- Mature individuals usually with dark lateral bands (figs. 14-15).

These characters clearly differentiate *A. lusitanicus* s.l. and *A. subfuscus* s.l. from *A. ater* s.l., for the latter species has the following character combination (fig. 13C) :

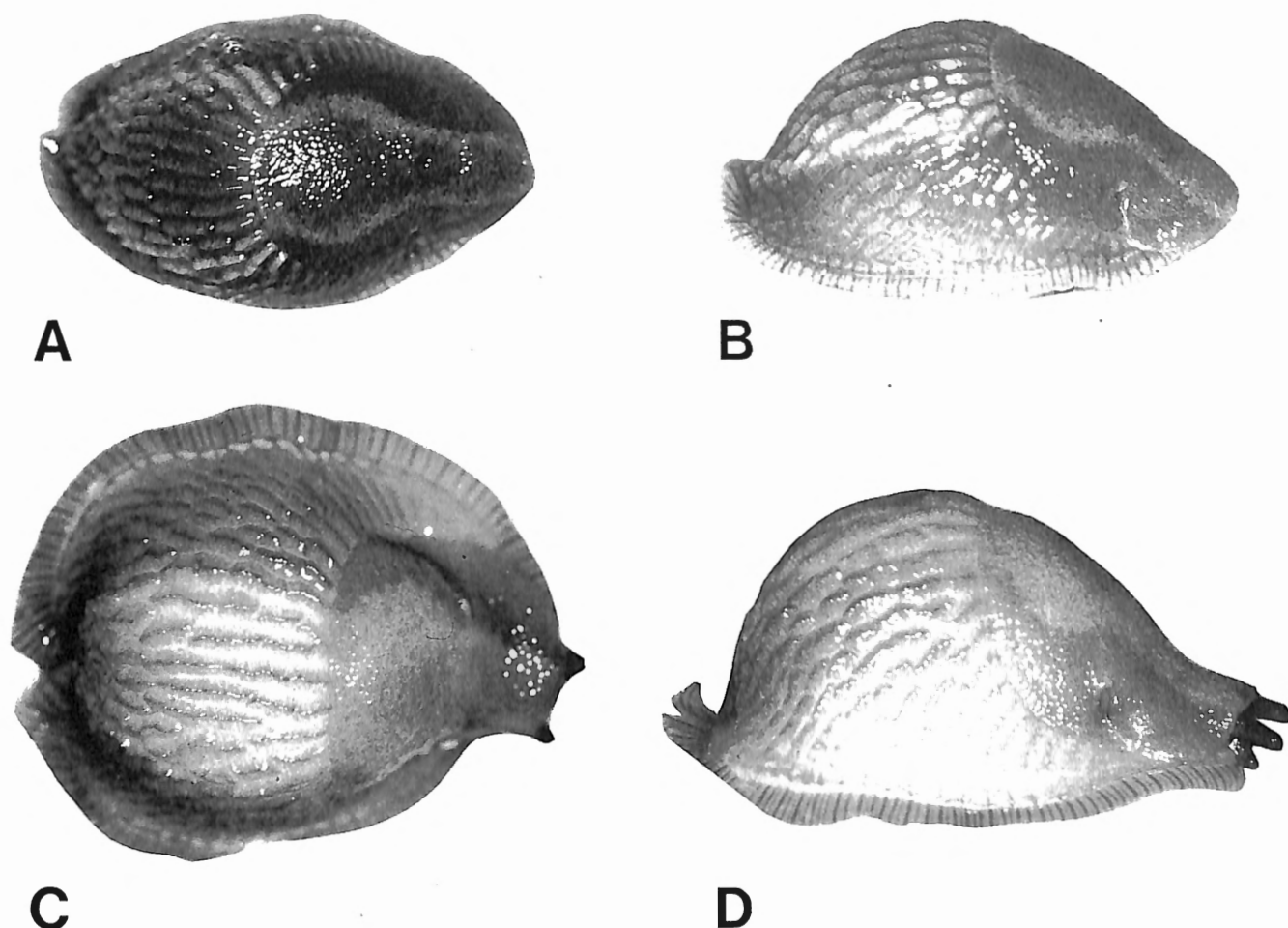


Fig. 15. – *Habitus* of *A. lusitanicus* s.l. - A-B : Thuès-Les-Bains, F (real size \pm 40 mm) - C-D : Aspet, F (real size \pm 50 mm) (see also BACKELJAU (1989b)).

- Genital atrium large with a strongly developed distal compartment in which the ligula is located.
- Oviduct short and not differentiated.
- The ligula is a large, bulky and knoblike papila.
- Mature individuals usually unbanded, even though juveniles sometimes have well-marked lateral bands (fig. 16).

There are also characters in which *A. lusitanicus* s.l. resembles *A. ater* s.l. The most important of these is the number and structure of the skin tubercles (figs. 14–16). The tubercles of *A. lusitanicus* s.l. are usually large, prominent and elongated as in *A. ater* s.l. (figs. 15–16), whereas those of *A. subfuscus* s.l. are smaller, flatter and more polygonal (fig. 14). Consequently, *A. subfuscus* s.l. has on the average more tubercles in cross section behind the mantle (fig. 14), than *A. lusitanicus* s.l. or *A. ater* s.l. (CAMERON *et al.*, 1983). Yet, there is a considerable intraspecific variation in the tubercle form of *A. lusitanicus* s.l. (fig. 15) (BACKELJAU, 1989b) and hence we do not use this feature to allocate the species to one or another species group.

We neither rely on the “rocking” behaviour (= swaying and twisting the contracted body when prodded) of *A. ater* s.l., for it seems that the occurrence of this behaviour varies

from one individual or population to another (DAVIES, 1987). As such it is not appropriate to use it in the present context: it is not because *A. lusitanicus* s.s. has once been observed to rock (DAVIES, 1987), that it would be more closely related to *A. ater* s.l., than to *A. flagellus* or *A. subfuscus* s.l., which both do not seem to rock (CAMERON *et al.*, 1983; DAVIES, 1987).

The colour of the mucus in *Arion* s.s. and *Mesarion* is also too variable to be a reliable group character. In *A. subfuscus* s.l., for example, some forms have a conspicuous orange-yellowish mucus, while in others it is colourless. A similar situation is found in *A. lusitanicus* s.l., for the mucus of *A. flagellus* is usually faintly yellow, while the mucus of *A. ater* s.l. and *A. lusitanicus* s.s. is colourless with sometimes an orange tinge in the latter species (CAMERON *et al.*, 1983).

In conclusion, for the time being we see no reasons to allocate *A. lusitanicus* s.s., *A. flagellus* and *A. subfuscus* s.l. to different species-groups (“subgenera”). Therefore, we include *A. lusitanicus* s.l. in *Mesarion* (RISCH & BACKELJAU, 1989; BACKELJAU, 1989b). This suggestion may be evaluated by checking the chromosome numbers of the involved species.



A



B

Fig. 16. – Habitus of *A. ater* s.l. - A : southern Sweden (real size ± 70 mm) - B : juvenile specimen from Antwerpen, B (real size ± 20 mm).

Towards a classification of *Arion*

As outlined earlier, there exist two current classifications of *Arion* spp. Firstly there is the widely adopted system of HESSE (1926) and there is the recent proposal of DAVIES (1987). The former system has been criticized, because the characters onto which it is based (appendix 4), are not convincing. Indeed, HESSE's (1926) classification is mainly based on features which are neither constant, nor restrictive, so that the delimitation of the subgenera remains confused.

The classification of DAVIES (1987) relies on two features, whose interpretation is subjective. The "subgeneric" differences between the spermatophores of *Mesarion*, *Arion* and *Carinarion*, for example, are not clear, since all these spermatophores are elongated and serrated (Pl. 1-5). However, several *Kobeltia* spermatophores are also serrated (figs. 3B and 4), but this was not mentioned by DAVIES (1987), who characterized the *Kobeltia* spermatophore by its anterior truncation. The latter feature does not apply to the *A. distinctus* spermatophore, which is tapering (fig.

3A). Thus the "subgeneric" differentiation of spermatophores in *Arion* is not unambiguous. Consequently, even though arionid spermatophores may be species specific, they are not so appropriate to distinguish taxa above the species level. Similarly the species specific spermatophores of Milacidae do not enable the distinction between species-groups or genera (*Milax* vs. *Tandonia*) (WIKTOR, 1987a, b). Nevertheless, spermatophores have been successfully used in the supraspecific systematics of Urocyclinae (VAN GOETHEM, 1977), some Pyramidellidae (ROBERTSON, 1978), Siphonariidae (HUBENDICK, 1947) and Neritidae (ANDREWS, 1937). Thus, perhaps that further studies of arionid spermatophores will reveal useful supraspecific characters too (e.g. the polygonal form of the *Carinarion* spermatophore, see Pl. 1-2).

The structure of the eggs and egg clutches on the other hand, is also an unreliable character for it is difficult to quantify objectively. It is for example not clear what should be considered as "sticky", "chalky", etc.

In conclusion, the classifications of HESSE (1926) and DAVIES (1987) are not satisfactory, because they are based on too variable and too vaguely defined characters. Yet, as we will point out further on, we do not refute a priori the species-groups distinguished by these authors.

The classification we provisionally adopt basically relies on the chromosome data of BEESON (1960). If *A. intermedius* is placed together with *A. hortensis* s.l., then each of the four species-groups of POLLONERA (1887) (fig.1) has a type species with a well-defined chromosome number. This feature has the advantage to be discretely quantified. Moreover, it allows testable predictions. For example, if *A. fagophilus* is a *Kobeltia*, then BACKELJAU & DE WINTER (1987) predicted for this species a haploid chromosome number of $n=28$. Similarly, RISCH & BACKELJAU (1989) suggested that if *A. lusitanicus* s.l. belongs to *Mesarion*, it is expected to have a haploid chromosome number of $n=25$.

This chromosome classification is also supported by biochemical, morphological and anatomical characteristics, which enable the distinction of two larger units (chromosome groups), each containing two species-groups. These two larger units reflect tendencies in chromosome numbers, electrophoretic characteristics of albumen gland proteins, the relative sizes of the slugs and the development of a ligula.

Thus, the currently available data corroborate POLLONERA's (1887) division of *Arion* (fig. 1). Our modified and updated version of this classification is outlined below. Measurements refer to live specimens.

Chromosome group : *Lochea*

Medium-sized to very large slugs (usually > 5 cm) with a complex and well-developed ligula. Haploid chromosome number : $n < 27$. The albumen gland proteins do not yield discrete fractions with PAGE (fig. 17). Contains the species-groups *Mesarion* and *Arion* s.s.

Species-group : *Mesarion*

Genital atrium relatively small with no, or a very weakly

developed, distal compartment. The oviduct is long (generally longer than half the length of epiphallus + vas deferens). Usually, its proximal portion is strongly developed for it contains a ligula consisting of longitudinal folds (fig. 13A-B). Adults generally with dark lateral bands (figs. 14-15). Haploid chromosome number: $n=25$. Type: *A. subfuscus*. Other tentative examples: *A. urbiae*, *A. lusitanicus*, *A. flagellus*, *A. anguloi*, *A. sibiricus*, *A. simrothi* and *A. magnus*.

Species-group: *Arion* s.s.

Genital atrium large with a strongly developed, conspicuous and muscled distal compartment. This compartment contains a massive, conical to knoblike ligula (fig. 13C), which never consists of longitudinal folds. The oviduct is short (generally shorter than half the length of epiphallus + vas deferens) and undifferentiated (fig. 13C). Adults only very rarely with dark lateral bands. Haploid chromosome number: $n=26$. Type: *A. ater*. Other examples: *A. rufus*.

Chromosome group: *Prolepis*

Small to medium-sized slugs (usually < 5cm), in which the ligula is absent or at least relatively inconspicuous, simple and weakly developed. Haploid chromosome number: $n>27$. The albumen gland proteins yield discrete and well-defined fractions with PAGE (fig. 7). Contains the species-groups *Kobeltia* and *Carinarion*.

Species-group: *Kobeltia*

Sole light yellowish to deep reddish orange (sometimes white). Back evenly rounded, without a fine mediodorsal row of lighter tubercles (not "keeled"). Mucus generally yellowish. Bursa of the spermatheca more or less globose, not tapering and without a pointed tip (fig. 4). Species monomorphic for the PGD₁₀₀ electromorph (fig. 18). Haploid chromosome number: $n=28$. Type: *A. hortensis*. Other tentative examples: *A. hortensis* s.s., *A. owenii*, *A. intermedius*, *A. alpinus* and *A. fagophilus*.

Species-group: *Carinarion*

Sole white. Back usually with a fine mediodorsal row of whitish tubercles, which mimic a faint "keel" (a real, but very weak keel is sometimes also visible). Mucus colourless and transparent. Bursa of the spermatheca pear-shaped to triangular, tapering and with a pointed tip (fig. 19). Species monomorphic for the PGD₈₆ electromorph (fig. 18). Haploid chromosome number: $n=29$. Type: *A. circumscriptus*. Other examples: *A. fasciatus* and *A. silvaticus*.

Several species assignments proposed here, are based on morphological and anatomical data and may therefore be regarded as predictions with respect to the involved chromosome numbers and electrophoretic characteristics.

Although our arrangement reflects a current state of knowledge, it does not provide a proper place for *A. franciscoi*. This species has a diverticulum near the junction of the epiphallus and the vas deferens (fig. 20). This is unique in the genus *Arion* and therefore it is likely that the species

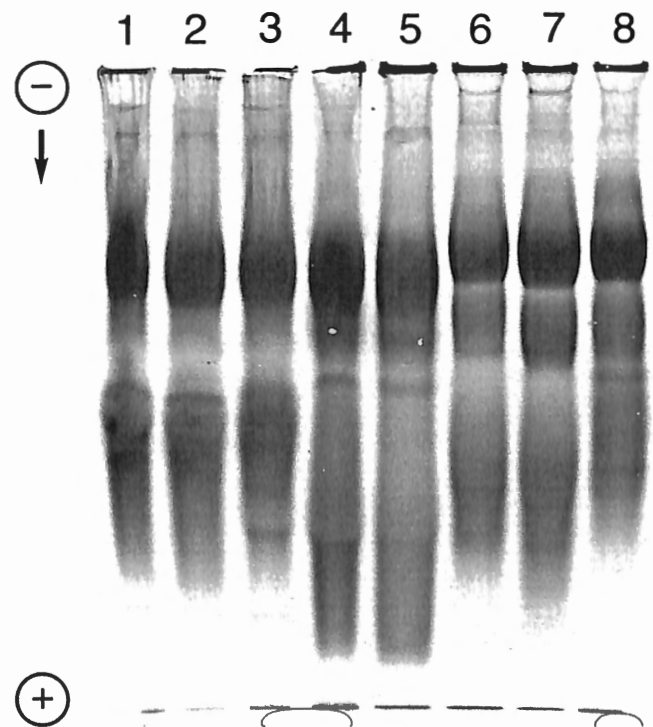


Fig. 17. - Discontinuous polyacrylamide gel electrophoresis profiles of albumen gland proteins in *A. ater* s.l. and *A. subfuscus* s.l. 1-3: *A. ater* s.l. (Boom, B); 4-5: *A. ater* s.l. (Bergen, N); 6-8: *A. subfuscus* s.l. (6: Renkum, NI; 7: Matagne-le-Petite, B; 8: Dinant, B) (modified after BACKELJAU & DE WINTER (1987)).

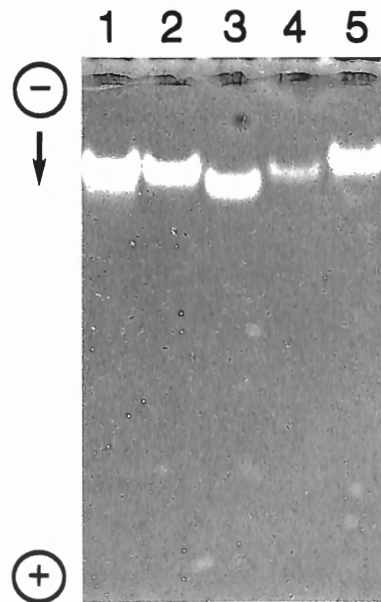


Fig. 18. - Continuous polyacrylamide gel electrophoresis profiles of PGD in five arionid species. 1. *A. distinctus* (Deurne-Antwerpen, B); 2. *A. intermedius* (Hamburg, D); 3. *Geomalacus maculosus* (Glengarriff, Ireland); 4. *A. fagophilus* (Alsasua, E); 5. *A. fasciatus* s.s. (Göteborg, S).

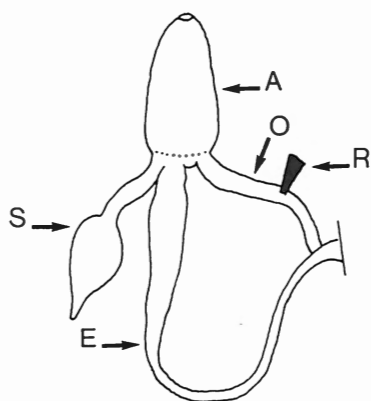


Fig. 19. – Simplified outline of the proximal genital parts in *A. fasciatus* s.l. Abbreviations as in figs. 4 and 13.

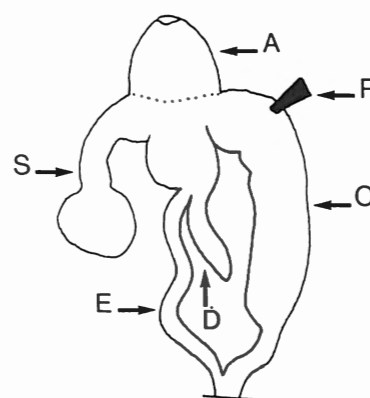


Fig. 20. – Simplified outline of the proximal genital parts in *A. franciscoi*. Abbreviations as in figs 4 and 13, adding *D* = diverticulum.

belongs to a still undefined species-group. The systematics of *A. franciscoi* will be discussed in a forthcoming paper. The four species-groups in our classification, are basically the same as those of HESSE (1926), at least if his sections *Kobeltia* and *Microarion* are fused. Yet, HESSE (1926) did not retain higher taxa comparable to our chromosome groups.

DAVIES (1987) not only recognized the same four species-groups as we, but also united *Arion* s.s. and *Mesarion* in a larger group. However, DAVIES (1987) gave *Kobeltia* and *Carinarion* the same rank as the compound group *Mesarion* + *Arion* s.s. In this way DAVIES (1987) implicitly adopted the arrangement of POLLONERA (1885), who also distinguished three equivalent species-groups viz. *Lochea*, *Prolepis* and *Carinella*.

These alternative opinions demonstrate the problem of attaching "taxonomic levels" to the different groups. Although we discern two chromosome groups and four species-groups, this does not necessarily imply that these groups are taxonomically equivalent. There is for example the fact that *Arion* s.s., *Mesarion* and *Kobeltia* share the PGD₁₀₀ electromorph, whereas *Carinarion* is characterized by PGD₈₆ (fig. 18). Since *Geomalacus maculosus* displays the PGD₁₁₄ electromorph (fig. 18) (BACKELJAU, 1989a), it can be argued that *Carinarion* deserves a higher rank than the three other species-groups.

On the other hand, the location of the ligula and the insertion of the oviducal retractor in *Lochea* (fig. 13) suggest that distal atrial compartment of *Arion* s.s. is the homologue of the proximal oviducal portion of *Mesarion* s.s. If this is correct, then the differences between *Arion* s.s. and *Mesarion* may be less fundamental than those between *Kobeltia* and *Carinarion* or than those between *Lochea* as a whole and the two latter species-groups. So, one can also postulate three (DAVIES, 1987), instead of four groups.

The problem of the taxonomic ranks can only be successfully dealt with after a sound phylogenetic analysis. Yet, such an approach requires a much more extended systematic knowledge about *Arion* than presently is available. Nevertheless, in the next section we will discuss some tentative phylogenetic and evolutionary considerations.

Evolutionary and phylogenetic considerations

If the correspondence between chromosome numbers and species groupings is confirmed, and if it is accepted that in euthyneuran molluscs higher chromosome numbers represent phylogenetically more advanced states (BURCH, 1965; PATTERSON & BURCH, 1978), then it follows that *Carinarion* ($n=29$) is the most recent species-group, whereas *Mesarion* ($n=25$) should be the oldest one (BACKELJAU & DE WINTER, 1987). However, it has been suggested that in eukaryotes chromosome fusions (= reduction of the chromosome number) are more common than chromosome fissions (= increasing chromosome numbers) (DOBZHANSKY *et al.*, 1977). This would reverse the phylogeny suggested above, but as pointed out by WHITE (1973), both types of chromosomal rearrangements intervene in speciation processes. Thus, a third phylogenetic hypothesis can be formulated, involving an ancestral form with $n=27$, giving rise to two lineages: one by chromosome fusions leading to *Lochea*, the other by chromosome fissions leading to *Prolepis*. The more ancient group in *Lochea* would then be *Arion* s.s. instead of *Mesarion*. Finally, one can also assume a polyphyletic origin of *Arion*. In that case, both *Lochea* and *Prolepis* may have undergone a parallel evolution towards higher chromosome numbers and a reduction of the free oviduct (figs. 4, 13).

Only the first two hypotheses have hitherto been advocated. SIMROTH (1885b) and TAYLOR (1907), for example, regarded *A. subfuscus* as respectively "die einfachsten unter dem Arionen" and "a more ancient species". SIMROTH (1885b) even suggested to relate *A. subfuscus* with *Geomalacus maculosus*, because *A. pegorarii* (a species which is usually considered to be a variety of *A. subfuscus*) has four dark bands over its back, a feature shared by *G. maculosus*.

The possible relationship between *Geomalacus* and *Mesarion* is still speculative. Once again, a chromosome analysis could be relevant here, for if *Geomalacus* is indeed closely related to, and older than, *Mesarion*, then its haploid chromosome number is expected to be $n \leq 25$. Note that the Philomycidae, which constitute an earlier (?)

branch of the ancestral stock from which the Arionidae arose (PILSBRY, 1948; RUNHAM & HUNTER, 1970; CHICHESTER & GETZ, 1973; LIKHAREV & WIKTOR, 1980), have a haploid chromosome number of $n=24$ (PATTERSON & BURCH, 1978). This observation is even more suggestive since TILLIER (1989) regarded the Philomycidae as a subfamily of the Arionidae.

The chromosome fusion hypothesis was implicitly advocated by QUICK (1960), who believed that rather *A. fasciatus* and *A. intermedius*, instead of *A. subfuscus*, would be the *Arion* species with more ancestral characters. Also SIMROTH (1891) regarded *A. intermedius* as an "Urform". This opinion was based on (1) the absence of a ligula in both species, (2) the limited eversion of the genitalia during coition and (3) the more anteriorly situated genital porus. According to QUICK (1960), *A. ater* would then be the most advanced form, because this species no longer displays dark lateral bands. This feature was also used by SIMROTH (1885a), who suggested that within both the Mono- and Diatriidae there would exist a tendency towards monochromatism.

At present we do not concur with QUICK's (1960) opinion. *A. fasciatus* s.l. combines so many special features such as 1) the bell-shaped body, 2) the whitish mediodorsal tubercle row, 3) the tapering and pointed bursa of the spermatheca (fig. 19), 4) the high chromosome number and 5) the predominant uniparental reproduction, that this species-group cannot be interpreted as an ancient branch. Moreover, the electrophoretic characteristics of *A. fasciatus* s.l. are usually also unique among the arionids hitherto studied (BACKELJAU & DE WINTER, 1987; BACKELJAU, 1989a). An example is the PGD₈₆ variant (fig. 18).

Although not much is known about the relationship between electrophoretic mobilities and the degree of dissimilarity between allozymes, it has been suggested that for a given protein, greater mobility differences between allozymes also imply greater structural differences (FERGUSON, 1980). This premise relies on the common observation that when enzyme profiles of related species are compared by standard electrophoretic methods, the electromorphs are distributed equidistantly. It is then assumed that a single mutation would correspond to a "unit of mobility difference" and so one could infer the number of presumed mutations needed to accomplish the observed mobility differences (JELNES, 1983). If this hypothesis is correct, more mutations are required to explain the mobility differences between the PGD electromorphs of *Geomalacus* and *A. fasciatus* s.l., than between those of *Geomalacus* and other *Arion* species (note the constant mobility difference of 14 units between PGD₈₆, PGD₁₀₀ and PGD₁₁₄). This supports the apomorphic character of the PGD₈₆ variant of *A. fasciatus* s.l.

Yet, the mobility criterion is controversial and probably not appropriate for all enzymes. SOD, for example, does not fit into this model, neither as it fits into the molecular clock hypothesis (AYALA, 1986). Hence, when phylogenetic relationships are inferred from electrophoretic data, it should be realized that different proteins have different evolutionary rates (NEI, 1987). Thus, slowly evolving pro-

teins are more informative for higher category systematics, whereas at the species level (or lower) faster evolving proteins are more relevant (NEI, 1987; HILLIS, 1989).

Altogether, the available data indicate that *A. fasciatus* s.l. should not be regarded as an ancient species-group. This is the more so since the presumed ancestral features of *A. fasciatus* s.l. (e.g. the simple oviduct and the limited eversion of the genitalia during copulation) (QUICK, 1960) can be interpreted as derived character states as well. This viewpoint will be expanded by referring to *A. intermedius*. *A. intermedius* has indeed also been considered as an ancient species, for its genitalia are comparatively simple (QUICK, 1960). This could be an ancestral configuration from which the genitalia of other *Arion* species were derived. Yet, earlier we already pointed out that the genital features of *A. intermedius* only reflect an uniparental breeding system and it seems unlikely that such a breeding system is an ancestral condition, especially not in proterandric hermaphrodites (IKEDA, 1937; SMITH, 1966; VISSER, 1977; but see LUCHTEL (1972) for the definition of proterandric hermaphroditism). On the contrary, uniparental reproductive modes are commonly regarded as recently acquired traits (HÄUSER, 1987).

The derived character state of the simple oviduct in *A. intermedius* can also be deduced from the observation that within the family Arionidae, there exists a tendency towards a gradual reduction of the copulatory organs. In more ancient genera such as *Ariopelta*, *Ariostralis*, *Binneya*, *Hemphillia*, and *Ariolimax* there is a well-developed penis (PILSBRY, 1898, 1948; PILSBRY & VANATTA, 1898; MEAD, 1943; SIRGEL, 1985; VISSER, 1988), whereas in presumably younger genera (e.g. *Anadenus*) this organ starts to reduce till it is eventually lost in *Arion* (PILSBRY, 1898, 1948; VISSER, 1988). The simple oviduct in *A. intermedius* and *A. fasciatus* s.l., may be a continuation of this tendency, for in these two species, not only the penis would have regressed, but also other copulatory genital parts such as the ligula and the proximal portion of the oviduct.

This assumption has been implicitly debated by WEBB (1961), who argued that (1) the genitalia of *Arion* do contain a strongly reduced, yet functional penis, (2) the arionid oviduct does not assume the function of a penis and (3) the genera *Binneya*, *Hemphillia*, *Ariolimax* and *Anadenus* (to name a few) do not belong to the Arionidae. With respect to point 3 most recent authors do not follow WEBB (1961) and hence we assign the genera mentioned above to the Arionidae. On the other hand, WEBB's (1961) opinion about the penis (or its remnants) in *Arion* may be partly correct (BACKELJAU & VAN BEECK, 1986; DE WINTER, 1986), while the copulatory function of the arionid oviduct is indeed still debated. Yet, the latter two points do not weaken the "genital reduction hypothesis".

This hypothesis is furthermore supported by the apparent relationship between the form of the spermatophore, the duration of the copulation, the development of the oviduct, and, eventually, the nature of the breeding system(s) in *Kobeltia*.

This is illustrated by the following comparison :

A. hortensis s.s.

Oviduct tripartite and well-developed. Its proximal eversible portion with a ligula-like structure. Spermatophore with a straight serrated ridge, an apical nozzle forming an angle of 90° with the longitudinal axis, a thick and rigid attachment collar and a strongly curved hook at the posterior end (figs. 3B and 4B). These features, and particularly the configuration of the apical nozzle and the posterior hook, may counteract the spermatophore transfert. The copulation lasts for at least 80 min.

A. owenii

Oviduct also tripartite, but the proximal eversible portion (with the ligula-like structure) is less developed than in *A. hortensis* s.s. (fig. 4C). The spermatophore is also similar to that of *A. hortensis* s.s., but its serrated ridge is spirally winded around the spermatophore, its apical nozzle points along the longitudinal axis and its posterior hook is less curved (fig. 4D). The alternative orientation of the apical nozzle and posterior hook may slightly reduce the resistance during spermatophore transfert. The copulation in *A. owenii* lasts for about 40-60 min.

A. distinctus

Oviduct dimorphic. A minority of specimens has a tripartite oviduct comparable to that of the two previous species (fig. 4E). The majority of specimens, however, has a bipartite oviduct, in which the proximal eversible portion (with the ligula-like structure) is strongly reduced or completely absent (fig. 4F). The elongated and fusiform spermatophore (figs. 3A and 4G) has no, or only very weak, serrations (fig. 21), while its attachment collar is poorly developed. Both the apical nozzle and the posterior hook, are tapering and point along the longitudinal axis of the spermatophore. This configuration may enhance spermatophore transfert. The copulation lasts for at most 30 min.

A. intermedius

Here the oviduct not only lost its proximal eversible portion, but also its firm portion (fig. 4H). We interpret this oviducal type as the homologue of the distal channel in the oviduct of *A. hortensis* s.l. This hypothesis is supported by the oviducal retractor of *A. hortensis* s.l. which is implanted near the junction of the firm portion of the oviduct and the distal channel. In *A. intermedius* the oviducal retractor inserts near the junction of the oviduct and the atrium (fig. 4). This configuration suggests a loss of the firm oviducal portion in *A. intermedius*, resulting in a direct connection between the distal channel of the oviduct and the atrium. This type of oviduct is probably no longer functional as copulatory organ and coition has been abandoned. Consequently, spermatophores are no longer produced.

Despite the puzzling existence of the oviducal dimorphism in *A. distinctus*, it seems as if the regression of the oviduct in *A. hortensis* s.l. proceeds parallel to a decrease in the eversion of the genitalia during mating (*A. hortensis* s.s. > *A. owenii* > *A. distinctus* with a bipartite oviduct), until

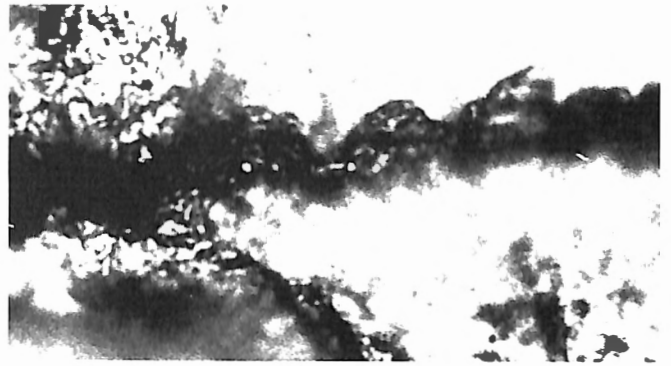


Fig. 21. – Light microscopic photograph (x125) of the "serrated" keel of the spermatophore in *A. distinctus* (Temse, B).

copulation eventually is abolished and the genitalia are no longer everted (*A. intermedius*). The degree of genital eversion is also expected to influence the duration of the copulation.

Given that internal fertilization is required as a precondition for the evolution of terrestrial gastropods (VISSER, 1988), it is evident that the abolition of copulation and spermatophore formation should be accompanied by a switch from biparental to uniparental reproduction. The evolutionary and ecogenetic consequences of such a transition, as well as its possible origin, will be expanded in a forthcoming paper about *A. fasciatus* s.l. Yet, if the "genital simplification hypothesis" is correct, one should also regard the limited eversion of the genitalia of *A. fasciatus* s.l. (GERHARDT, 1935) as a derived character state, related to the reduction of the oviduct in this species-group.

One could also presume a link between the development of the serrated keels on the spermatophores and the duration of copulations. The denticles point indeed towards the apical side of the spermatophore (fig. 3, Pl. 1-5) and could thus hinder spermatophore transfert. Consequently, the stronger the dentitions, the longer the copulation would last. Note, that the degree of dentition not only varies between individuals but also between different parts of single spermatophores, in which for example the apical side is usually less serrated than the distal regions (Pl. 1-5).

However, DAVIES (1987) remarked that during their transfert, spermatophores are covered by a thick mucus layer which prevents any possible resistance provoked by the denticles. Therefore, DAVIES (1987) regarded the serrated keels as reinforcing structures. If this is correct, one should expect that the most fragile spermatophores must bear the strongest keels. Yet, this is not the case since precisely the most fragile spermatophores (e.g. *A. distinctus*) have no significant keels at all.

This observation supports KÜNKEL's (1916) suggestion that the serrated keels facilitate the rupture of the spermatophores, for the ridges are less flexible than the rest of the spermatophore wall. As a consequence, this latter is ruptured along the ridges when the spermatophore is moved and curved in the recipient genitalia. The orientation of

the serrations may enhance this process by hindering the spermatophore movements so that the tension in the spermatophore wall is increased (the mucus cover, which surrounds the spermatophore during transfert, is assumed to be digested or detached in the recipient genitalia). The more fragile spermatophore of *A. distinctus*, on the contrary, may be weak enough to be ruptured without the intervention of tension enforcing structures. This is of course a hypothesis. In *Helix pomatia*, for example, spermatophores should not be ruptured to release spermatozoa, because they have a small porus at their distal end. In this case the ridges would enhance elastic resistance (LIND, 1973).

Altogether, despite the hypothetical character of several viewpoints outlined in this section, we conclude that the simple oviduct and the uniparental reproduction in *A. intermedius* and *A. fasciatus* s.l. are derived features, rather

than primitive ones. Therefore, we provisionally regard both species as recent representants of the genus *Arion*.

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References

- ADAMS, L.E., 1910. Observations on the pairing of *Arion ater* (L.). *Journal of Conchology*, 13 : 116-119.
- ALBONICO, R., 1948. Die Farbvarietäten der grossen Wegschnecken, *Arion empiricorum* Fér., und deren Abhängigkeit von der Umweltbedingungen. *Revue Suisse de Zoologie*, 55 : 347-425.
- ANDREWS, E.A., 1937. Certain reproductive organs in the Neritidae. *Journal of Morphology*, 61 : 525-561.
- ANT, H., 1957. Westfälische Nacktschnecken. *Natur und Heimat*, 17 : 1-20.
- ARCHIE, J.W., SIMON, C. & MARTIN, A., 1989. Small sample size does decrease the stability of dendrograms calculated from allozyme-frequency data. *Evolution*, 43 : 678-683.
- AYALA, F.J., 1986. On the virtues and pitfalls of the molecular evolutionary clock. *Journal of Heredity*, 77 : 226-235.
- BACKELJAU, T., 1981. Biometrie, ecologie, ethologie en systematiek van het *Arion hortensis* complex in België. Unpublished "licentiaatsverhandeling", Universitaire Instelling Antwerpen, 78 pp.
- BACKELJAU, T., 1985. Estimation of genic similarity within and between *Arion hortensis* s.l. and *A. intermedius* by means of isoelectric focused esterase patterns in hepatopancreas homogenates (Mollusca, Pulmonata : Arionidae). *Zeitschrift für zoologische Systematik und Evolutionsforschung*, 23 : 38-49.
- BACKELJAU, T., 1987. Electrophoretic distinction between *Arion hortensis*, *A. distinctus* and *A. owenii* (Mollusca : Pulmonata). *Zoologischer Anzeiger*, 219 : 33-39.
- BACKELJAU, T., 1989a. Systematische studie van enkele Westeuropese Arionidae (Mollusca, Pulmonata). Unpublished Ph. D. Thesis, Universitaire Instelling Antwerpen, 299 pp.
- BACKELJAU, T. 1989b. The original diagnoses of *Arion simrothi* KÜNDEL and *A. magnus* TORRES MINGUEZ (Mollusca, Pulmonata). *Annales de la Société Royale Zoologique de Belgique*, 119 : 199-211.
- BACKELJAU, T. & DE WINTER, A.J., 1987. An electrophoretic characterisation of three paratypes of *Arion fagophilus* DE WINTER, 1986, with notes on the subgeneric division of the genus *Arion* FÉRUSAC, 1819 (Mollusca, Pulmonata). *Zeitschrift für zoologische Systematik und Evolutionsforschung*, 25 : 169-180.
- BACKELJAU, T. & MARQUET, R., 1985. An advantageous use of multivariate statistics in a biometrical study on the *Arion hortensis* complex (Pulmonata : Arionidae) in Belgium. *Malacological Review*, 18 : 57-72.
- BACKELJAU, T. & VAN BEECK, M., 1986. Epiphallus anatomy in the *Arion hortensis* species aggregate (Mollusca, Pulmonata). *Zoologica Scripta*, 15 : 61-68.
- BACKELJAU, T., AHMADYAR, S.Z., SELENS, M., VAN ROMPAEY, J. & VERHEYEN, W., 1987. Comparative electrophoretic analyses of three European *Carinarion* species (Mollusca, Pulmonata : Arionidae). *Zoologica Scripta*, 16 : 209-222.
- BACKELJAU, T., DAVIES, S.M. & DE BRUYN, L. 1988. An albumen gland protein polymorphism in the terrestrial slug *Arion owenii*. *Biochemical Systematics and Ecology*, 16 : 425-429.
- BEESON, G.E., 1960. Chromosome numbers of slugs. *Nature*, 186 : 257-258.
- BOATO, A., 1986. A preliminary karyological analysis of five species of *Solatopupa* (Pulmonata, Chondrinidae). *Bollettino di Zoologia*, 53 : 15-22.
- BOATO, A., BODON, M. & GIUSTI, F., 1983. On a new slug from the Northern Apennines (Pulmonata : Arionidae). *Archiv für Molluskenkunde*, 114 : 35-44.
- BOETTGER, C.R., 1949. Zur Kenntnis der grossen Wegschnecken (*Arion* s. str.) Deutschlands. *Archiv für Molluskenkunde*, 78 : 169-186.
- BURCH, J.B., 1961. The chromosomes of *Planorbarius corneus* (LINNAEUS) with a discussion on the value of chromosome numbers in snail systematics. *Bacteria*, 25 : 45-51.
- BURCH, J.B., 1965. Chromosome numbers and systematics in Euthyneuran snails. *Proceedings of the First European Malacological Congress*, 215-241.
- BUTOT, L.J.M., 1967. Phylogenetic position of Heterurethra (Gastropoda : Euthyneura) in the light of cytotaxonomy. *Genen en Phaenen*, 11 : 53-55.

- BUTOT, L.J.M. & KIAUTA, B., 1967. The chromosomes of *Catinella arenaria* (BOUCHARD-CHANTEREAUX, 1837) with a review of the cytological conditions within the genus *Catinella* and considerations of phylogenetic position of the Succineoidea ord. nov. (Gastropoda : Euthyneura). *Beaufortia*, 14 : 157-164.
- BUTOT, L.J.M. & KIAUTA, B., 1969. Cytotaxonomic observations in the stylommatophoran family Helicidae, with considerations on the affinities within the family. *Proceedings of the Third European Malacological Congress, Malacologia*, 9 : 261-262.
- CAMERON, R.A.D., EVERS HAM, B. & JACKSON, N., 1983. A field key to the slugs of the British Isles. *Field Studies*, 5 : 807-824.
- CHEVALLIER, H., 1972. Arionidae (Mollusca, Pulmonata) des Alpes et du Jura français. *Haliotis*, 2 : 7-23.
- CHICHESTER, L.F. & GETZ, L.L., 1973. The terrestrial slugs of northeastern North America. *Sterkiana*, 51 : 11-42.
- COCKERELL, T.D.A., 1891a. Notes on *Arion hortensis*, *A. circumscriptus*, and their allies. *The Conchologist*, 1 : 49-52.
- COCKERELL, T.D.A., 1891b. *Arion circumscriptus*, JOHNSTON = *Bourguignati*, MAB. *The British Naturalist*, jan. 1891 : 20.
- COCKERELL, T.D.A., 1892. A check-list of the slugs. *The Conchologist*, 2 : 168-232.
- COLLINGE, W.E., 1892a. A review of the Arionidae of the British Isles. *The Conchologist*, 2 : 56-66.
- COLLINGE, W.E., 1892b. A review of the Arionidae of the British Isles (part 2). *The Conchologist*, 2 : 76-83.
- COLLINGE, W.E., 1893. On the structure and affinities of some European slugs. *The Conchologist*, 2 : 113-117.
- COLLINGE, W.E., 1897. On some European slugs of the genus *Arion*. *Proceedings of the Zoological Society, London*, 3 : 439-450.
- COYNE, J.A., EANES, W.F., RAMSHAW, J.A.M. & KOEHN, R.K., 1979. Electrophoretic heterogeneity of α -glycerophosphate dehydrogenase among many species of *Drosophila*. *Systematic Zoology*, 28 : 164-175.
- DAVIES, S.M., 1977. The *Arion hortensis* complex with notes on *A. intermedius* NORMAND (Pulmonata : Arionidae). *Journal of Conchology*, 29 : 173-187.
- DAVIES, S.M., 1979. Segregates of the *Arion hortensis* complex (Pulmonata : Arionidae), with the description of a new species, *Arion owenii*. *Journal of Conchology*, 30 : 123-127.
- DAVIES, S.M., 1987. *Arion flagellus* COLLINGE and *A. lusitanicus* MABILLE in the British Isles : a morphological, biological and taxonomic investigation. *Journal of Conchology*, 32 : 339-354.
- DE WILDE, J.J., 1983. Notes on the *Arion hortensis* complex in Belgium (Mollusca, Pulmonata : Arionidae). *Annales de la Société Royale Zoologique de Belgique*, 113 : 87-96.
- DE WINTER, A.J., 1984. The *Arion hortensis* complex (Pulmonata : Arionidae) : designation of types, descriptions, and distributional patterns, with special reference to the Netherlands. *Zoologische Mededelingen, Leiden*, 59 : 1-17.
- DE WINTER, A.J., 1986. Little known and new south-west European slugs (Pulmonata : Agriolimacidae, Arionidae). *Zoologische Mededelingen, Leiden*, 60 : 135-158.
- DOBZHANSKY, T., AYALA, F., STEBBINS, G.L. & VALENTINE, J.W., 1977. Evolution. W.H. Freeman & Company, San Francisco, 572 pp.
- DOLAN, S. & FLEMING, C.C., 1988. Isoenzymes in the identification and systematics of terrestrial slugs of the *Arion hortensis* complex. *Biochemical Systematics and Ecology*, 16 : 195-198.
- EHRMANN, P., 1933. Mollusca (Weichtiere). In : BROHMER, P., EHRMANN, P. & ULMER, G., Die Tierwelt Mitteleuropas, Band II, Lieferung 1. Verlag Von Quelle & Meyer, Leipzig, 264 pp.
- EVANS, N.J., 1983. Notes on self-fertilization and variation in body colour in *Limax flavus* L. and *L. pseudoflavus* EVANS. *The Irish Naturalists' Journal*, 21 : 37-40.
- EVERS HAM, B. & JACKSON, N., 1982. Slugs : a key for amateurs & professionals. Durham, privately published by N. Jackson, ISBN 0950732516, unpagged.
- FERGUSON, A., 1980. Biochemical systematics and evolution. Blackie, Glasgow/London, 194 pp.
- FÉRUS SAC, J.B.L. D'AUDEBARD de, 1819. Histoire naturelle générale et particulière des mollusques terrestres et fluviatiles. 2. Paris, 96 pp.
- FÉRUS SAC, J.B.L. D'AUDEBARD de, 1821. Tableaux systématiques des animaux mollusques classés en familles naturelles, dans lesquels on établit la concordance de tout les systèmes; suivis d'un prodrome général pour tous les mollusques terrestres ou fluviatiles, vivants ou fossiles. Arthus Bertrand, Paris, 114 pp.
- FOCARDI, S. & QUATTRINI, D., 1972. Structure of the reproductive apparatus and life cycle of *Milax gagates* (DRAPARNAUD). Mollusca Gastropoda Pulmonata. *Bollettino di Zoologia*, 39 : 9-27.
- FOLTZ, D.W., OCHMAN, H., JONES, J.S., EVANGELISTI, S.M. & SELANDER, R.K., 1982a. Genetic population structure and breeding systems in arionid slugs (Mollusca : Pulmonata). *Biological Journal of the Linnean Society*, 17 : 225-241.
- FOLTZ, D.W., SCHAITKIN, B.M. & SELANDER, R.K., 1982b. Genetic disequilibrium in the self-fertilizing slug *Deroceras laeve*. *Evolution*, 36 : 80-85.
- FOLTZ, D.W., OCHMAN, H. & SELANDER, R.K., 1984. Genetic diversity and breeding systems in terrestrial slugs of the families Limacidae and Arionidae. *Malacologia*, 25 : 593-605.
- FORCART, L., 1965. Rezenten Land- und Süßwassermollusken der suditalienischen Landschaften Apulien, Basilicata und Calabrien. *Verhandlungen der Naturforschenden Gesellschaft in Basel*, 76 : 59-184.
- GAIN, W.A., 1892. Some remarks on the colour changes in *Arion intermedius*, NORMAND. *The Conchologist*, 2 : 55-56.
- GERHARDT, U., 1935. Weitere Untersuchungen zur Kopulation der Nacktschnecken. *Zeitschrift für Morphologie und Ökologie der Tiere*, 30 : 297-332.
- GERHARDT, U., 1940. Neue Biologische Nacktschneckenstudien. *Zeitschrift für Morphologie und Ökologie der Tiere*, 36 : 557-580.
- GERMAIN, L., 1930. Faune de France 21. Mollusques terrestres et fluviatiles (première partie). Lechevallier, Paris, 447 pp.
- GITTENBERGER, E., BACKHUYS, W. & RIPKEN, T.E.J., 1984. De landslakken van Nederland (2de ed.). Uitgave van de Koninklijke Nederlandse Natuurhistorische Vereniging, No. 34, Leiden, 184 pp.
- GROSSU, A.V., 1983. Gastropoda Romaniae. 4. Ordo Stylommatophora. Suprafam. : Arionacea, Zonitacea, Ariophantacea si Helicacea. Editura Litera, Bucuresti, 563 pp.

- HÄUSER, C.L., 1987. The debate about the biological species concept. A review. *Zeitschrift für zoologische Systematik und Evolutionsforschung*, 25 : 241-257.
- HESSE, P., 1926. Die Nacktschnecken der palaearktischen Region. *Abhandlungen des Archiv für Molluskenkunde*, 2 : 1-152.
- HEYNEMANN, D.F., 1870. Vorläufiges Verzeichniss der Nacktschnecken Europas. *Nachrichtsblatt der Deutschen malakozoologischen Gesellschaft*, 2 : 162-165.
- HILLIS, D.M., 1989. Genetic consequences of partial self-fertilization on populations of *Liguus fasciatus* (Mollusca : Pulmonata : Bulimulidae). *American Malacological Bulletin*, 7 : 7-12.
- HILLIS, D.M., ROSENFELD, D.S. & SANCHEZ, M. Jr., 1987. Allozygic variability and heterozygote deficiency within and among morphologically polymorphic populations of *Liguus fasciatus* (Mollusca : Pulmonata : Bulimulidae). *American Malacological Bulletin*, 5 : 153-157.
- HOFFMANN, R.J., 1983. The mating system of the terrestrial slug *Deroceras laeve*. *Evolution*, 37 : 423-425.
- HUBENDICK, B., 1947. Phylogenie und Tiergeographie der Siphonariidae. Zur Kenntnis der Phylogenie in der Ordnung Basommatophora und des Ursprungs der Pulmonatengruppe. *Zoologiska Bidrag från Uppsala*, 24 : 1-216.
- IKEDA, K., 1937. Cytogenetic studies on the self-fertilization of *Philomycus bilineatus* BENSON. *Journal of Science of the Hiroshima University, Serie B, Division 1 (Zoology)*, 5 : 67-123.
- JELNES, J.E., 1983. A method of obtaining indices of distance and similarity from observations on differences in electrophoretic mobility of population samples. *Hereditas*, 98 : 281-286.
- KERNEY, M.P., CAMERON, R.A.D. & JUNGBLUTH, J.H., 1983. Die Schnecken Mitteleuropas. Paul Parey, Hamburg, 384 pp.
- KOFLER, A., 1977. Nachtrag zur Faunistik der Weichtiere (Mollusca) Osttirols. *Bericht des Naturwissenschaftlich-medizinischen Vereins in Innsbruck*, 64 : 67-87.
- KOFLER, A., 1986. Zweiter Nachtrag zur Faunistik der Weichtiere Osttirols (Mollusca). *Bericht des Naturwissenschaftlich-medizinischen Vereins in Innsbruck*, 73 : 71-86.
- KÜNKEL, K., 1916. Zur Biologie der Lungenschnecken. Carl Winters Universitätsbuchhandlung, Heidelberg, 440 pp.
- LEHMANN, R., 1862. Die Nacktschnecken aus der Umgebung Stettins und in Pommern. *Malakozoologische Blätter*, 9 : 156-193.
- LEHMANN, R., 1873. Die lebenden Schnecken und Muscheln der Umgegend Stettins und in Pommern mit besonderer Berücksichtigung ihres anatomischen Baues. Cassel, 328 pp.
- LESSONA, M., 1881. Sugli *Arion* del Piemonte. *Atti della Reale Accademia delle Scienze di Torino*, 16 : 185-197.
- LESSONA, M. & POLLONERA, C., 1882. Monografia dei limacidi italiani. *Atti della Reale Accademia delle Scienze di Torino*, 35 : 1-77.
- LIKHAREV, I.M. & WIKTOR, A., 1980. The fauna of the slugs of the USSR and adjacent countries (Gastropoda terrestria nuda) (in Russian). Akademia Nauk SSSR, Leningrad, 437 pp.
- LIND, H., 1973. The functional significance of the spermatophore and fate of spermatozoa in the genital tract of *Helix pomatia* (Gastropoda : Stylommatophora). *Journal of Zoology, London*, 169 : 39-64.
- LINNAEUS, C., 1758. Systema naturae. Tom. I. Ed. decima reformata. Holmiae, 824 pp.
- LINNAEUS, C., 1761. Fauna Svecica. Stockholmiae, 578 pp.
- LINNAEUS, C., 1767. Systema naturae. Tom. I. Pars II. Ed. duodecima reformata. Holmiae, 1069-1327.
- LOENS, H., 1890. Zum Formenkreis des *Arion subfuscus* DRAPARNAUD. *Nachrichtsblatt der Deutschen malakozoologischen Gesellschaft*, 22 : 155-161.
- LUCHTEL, D., 1972. Gonadal development and sex determination in pulmonate Molluscs. I. *Arion circumscriptus*. *Zeitschrift für Zellforschung und mikroskopische Anatomie*, 130 : 279-301.
- MABILLE, M.J., 1868. Des limaciens européens. *Revue et Magazine de Zoologie (2e serie)*, 20 : 129-146.
- MABILLE, M.J., 1870. Des limaciens français. *Annales de Malacologie*, 1 : 105-144.
- MANGANELLI, G. & GIUSTI, F., 1988. Notulae Malacologicae XXXVII. New data on *Arion intermedius* NORMAND in Italian Apennines and major Tyrrhenian Islands (Pulmonata : Arionidae). *Archiv für Molluskenkunde*, 119 : 39-54.
- MARTENBACH, L., 1940. Über den Farbwechsel von *Arion (Lochea) empiricorum* (FÉRUSAC, 1819). *Zeitschrift für wissenschaftliche Zoologie*, 152 : 473-506.
- MARTÍN, R., 1985. Los limacos del País Vasco y zonas adyacentes (Mollusca, Gastropoda : Agriolimacidae, Limacidae, Milacidae, Arionidae, Testacellidae). Unpublished "Tesis de licenciado", Universidad del País Vasco, 132 pp.
- MARTÍN, R. & GÓMEZ, B.J., 1988. A new slug from the Iberian peninsula : *Arion anguloi* n.sp. *Archiv für Molluskenkunde*, 118 : 167-174.
- MCCRACKEN, G.F. & BRUSSARD, P.F., 1980. Self-fertilization in the white-lipped land snail *Triodopsis albolabris*. *Biological Journal of the Linnean Society*, 14 : 429-434.
- MCCRACKEN, G.F. & SELANDER, R.K., 1980. Self-fertilization and monogenic strains in natural populations of terrestrial slugs. *Proceedings of the national Academy of Sciences of the U.S.A.*, 77 : 684-688.
- MEAD, A.R., 1943. Revision of the giant West coast land slugs of the genus *Ariolimax* MOERCH (Pulmonata : Arionidae). *American Midland Naturalist*, 30 : 675-717.
- MERMOD, G., 1930. Catalogue des invertébrés de la Suisse. Fascicule 18. Gastéropodes. Muséum d'Histoire Naturelle de Genève, 583 pp.
- MINICHEV, Y.S., 1974. Chromosome numbers and systematics of gastropods (in Russian). *Zoologicheskoy Zhurnal*, 53 : 1255-1257.
- MOQUIN-TANDON, A., 1855. Histoire naturelle des mollusques terrestres et fluviatiles de France. Tome second (+ atlas). J.-B. Bailliere, Paris, 664 pp.
- MURRAY, J. & CLARKE, B., 1976a. Supergenes in polymorphic land snails. I. *Partula taeniata*. *Heredity*, 37 : 253-269.
- NEI, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89 : 583-590.
- NEI, M., 1987. Molecular evolutionary genetics. Columbia University Press, New York, 512 pp.
- NICKLAS, N.L. & HOFFMANN, R.J., 1981. Apomictic parthenogenesis in a hermaphroditic terrestrial slug, *Deroceras laeve* (MÜLLER). *Biological Bulletin*, 160 : 123-135.
- ÖKLAND, F., 1922. Arionidae of Norway. *Skrifter utgitt av det Norske videnskapsakademi i Oslo. I. Mat.-Nat. Klasse*, 5 : 1-61.

- OLDHAM, C., 1942. Notes on *Geomalacus maculosus*. *Proceedings of the malacological Society, London*, 25 : 10-11.
- PATTERSON, C.M. & BURCH, J.B., 1978. Chromosomes of Pulmonate molluscs. pp. 171-217 in : FRETTER, V. & PEAKE, J. (eds.), *Pulmonates vol. 2A. Systematics, Evolution and Ecology*. Academic Press, New York, 540 pp.
- PERROT, J.L., 1939. Contribution à l'étude de la fécondation chez les pulmonés stylommatophores. Le cas de l'*Arion empiricorum* FÉR. (*Arion rufus* L.). *Bulletin Biologique de la France et de la Belgique*, 73 : 408-432.
- PILSBRY, H.A., 1898. Phylogeny of the genera of Arionidae. *Proceedings of the malacological Society, London*, 3 : 94-104.
- PILSBRY, H.A., 1948. Land Mollusca of North America (North of Mexico). Vol.II. Part 2. Monographs of the Academy of Natural Sciences of Philadelphia, 3 : 521-1113.
- PILSBRY, H.A. & VANATTA, E.G., 1898. Revision of the North American slugs : *Binneya*, *Hemphillia*, *Hesperarion*, *Prophysaon* and *Anadenulus*. *Proceedings of the Academy of Natural Sciences of Philadelphia* (no volume number), 219-262.
- PLATTS, E.A. & SPEIGHT, M.C.D., 1988. The taxonomy and distribution of the Kerry slug *Geomalacus maculosus* ALLMAN, 1843 (Mollusca : Arionidae) with a discussion of its status as a threatened species. *The Irish Naturalists' Journal*, 22 : 417-430.
- POLLONERA, C., 1885. Elenco dei Molluschi terrestri viventi in Piemonte. *Atti della Reale Accademia delle Scienze di Torino*, 20 : 3-31.
- POLLONERA, C., 1887. Specie nuove o mal conosciute di *Arion* europei. *Atti della Reale Accademia delle Scienze di Torino*, 22 : 290-313.
- POLLONERA, C., 1889. Nuove contribuzioni allo studio degli *Arion* europei. *Atti della Reale Accademia delle Scienze di Torino*, 24 : 401-418.
- POLLONERA, C., 1890. Recensement des Arionidae de la région Paléarctique. *Bollettino dei Musei di zoologia e di anatomia comparata della Reale Università di Torino*, 87 : 1-40.
- QUATTRINI, D., 1970. La riproduzione di *Milax gagates* (DRAPARNAUD). Mollusca Gastropoda Pulmonata. *Bollettino della Società italiana di biologia sperimentale*, 46 : 802-804.
- QUICK, H., 1946. The mating process in *Arion hortensis*, FÉRUSAC and in *Arion subfuscus*, DRAPARNAUD. *Journal of Conchology*, 22 : 178-182.
- QUICK, H.E., 1960. British slugs (Pulmonata; Testacellidae, Arionidae, Limacidae). *Bulletin of the British Museum of natural History (Zoology)*, 6 : 103-226.
- RAINER, M., 1967. Chromosomenuntersuchungen an Gastropoden (Stylommatophora). *Malacologia*, 5 : 341-373.
- RAMSHAW, J.A., COYNE, J.A. & LEWONTIN, R.C., 1979. The sensitivity of gel electrophoresis as a detector of genetic variation. *Genetics*, 93 : 1019-1037.
- RISCH, P. & BACKELJAU, T., 1989. On the occurrence of *Arion lusitanicus* MABILLE, 1868 in Belgium (Mollusca : Pulmonata). *Annales de la Société Royale Zoologique de Belgique*, 118 : 25-38.
- ROBERTSON, R., 1978. Spermatophores of six eastern North American pyramidellid gastropods and their systematic significance (with the new genus *Boonea*). *Biological Bulletin*, 155 : 360-382.
- ROBERTSON, R., 1989. Spermatophores of aquatic non-stylommatophoran gastropods : A review with data on *Heliacus* (Architectonidae). *Malacologia*, 30 : 341-364.
- RUNHAM, N.W. & HUNTER, P.J., 1970. *Terrestrial slugs*. Hutchinson University Library, London, 184 pp.
- SEIBERT, H., 1873. Die colorirten Tafeln des Lehmann'schen Werkes. *Nachrichtsblatt der Deutschen malakozoologischen Gesellschaft*, 6 : 79-82.
- SELANDER, R.K. & KAUFMAN, D.W., 1973. Self-fertilization and genetic population structure in a colonizing land snail. *Proceedings of the national Academy of Sciences of the U.S.A.*, 70 : 1186-1190.
- SELANDER, R.K. & OCHMAN, H., 1983. The genetic structure of populations as illustrated by molluscs. pp. 93-123 in : RATTAZZI, M.C., SCANDALIOS, J.C. & WHITT, G.S. (eds), *Isozymes : Current topics in biological and medical research*. Vol. 10. Genetics and evolution.
- SHELLEY, A.A. & CHASE, R., 1988. Courtship and copulation in the terrestrial snail *Helix aspersa*. *Canadian Journal of Zoology*, 66 : 1446-1453.
- SHERBORN, C.D., 1924. Index animalium. Part. V (pp. 945-1196). Index c-ceyl. Trustees of the British Museum, London.
- SIMROTH, H., 1884. Die deutschen Arionarten und ihre Färbung. *Sitzungsberichte der Naturforschenden Gesellschaft zu Leipzig*, (no volume number) : 19-22.
- SIMROTH, H., 1885a. Versuch einer Naturgeschichte der deutschen Nacktschnecken und ihrer europäischen Verwandten. *Zeitschrift für wissenschaftliche Zoologie*, 42 : 203-366.
- SIMROTH, H., 1885b. Ueber den *Geomalacus maculosus* Allm. *Sitzungsberichte der Naturforschenden Gesellschaft zu Leipzig*, (no volume number) : 12-13.
- SIMROTH, H., 1887. Einige Bemerkungen, betreffend die Systematik der europäischen Nacktschnecken. *Nachrichtsblatt der Deutschen malakozoologischen Gesellschaft*, 19 : 161-168.
- SIMROTH, H., 1891. Die Nacktschnecken der portugiesisch-azorischen Fauna. *Nova acta Academiae Caesareae Leopoldino Carolinae germanicae naturae curiosorum*, 56 : 201-424.
- SINGH, R.S., 1979. Genic heterogeneity within electrophoretic "alleles" and the pattern of variation among loci in *Drosophila pseudoobscura*. *Genetics*, 93 : 997-1018.
- SIRGEL, W.F., 1985. A new subfamily of Arionidae (Mollusca, Pulmonata). *Annals of the Natal Museum*, 26 : 471-487.
- SMITH, B.J., 1966. Maturation of the reproductive tract of *Arion ater* (Pulmonata : Arionidae). *Malacologia*, 4 : 325-349.
- SWOFFORD, D.L. & SELANDER, R.B., 1981. BIOSYS-1 : a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *The Journal of Heredity*, 72 : 281-283.
- TAYLOR, J.W., 1907. *Monograph of the land- & freshwater Mollusca of the British Isles*. Taylor Brothers, Leeds, 299 pp.
- TILLIER, S., 1989. Comparative morphology, phylogeny and classification of land snails and slugs (Gastropoda : Pulmonata : Stylommatophora). *Malacologia*, 30 : 1-303.
- TORRES MINGUEZ, A., 1925. Notas Malacológicas. VII. Cuatro nuevos *Arion* ibéricos y dos nuevos limacidos de Guinea (dibujos del autor). *Butletti de la Institucio Catalana d'Historia Natural*, Serie 2, Vol. 5, Núm. 8 : 228-243.

- TURTON, W., 1840. A manual of the land and freshwater shells of the British Islands, with figures of each of the kinds (2nd Ed. by J.E. GRAY). London, 324 pp.
- VAN GOETHEM, J.L., 1977. Révision systématique des Urocyclinae (Mollusca, Pulmonata, Urocyclidae). *Annales du Musée Royal de l'Afrique Centrale (8°), Sciences Zoologiques*, 218 : 1-355.
- VISSER, M.H.C., 1977. The morphology and significance of the spermoviduct and prostate in the evolution of the reproductive system of the Pulmonata. *Zoologica Scripta*, 6 : 43-54.
- VISSER, M.H.C., 1988. The significance of terminal duct structures and the role of neoteny in the evolution of the reproductive system of Pulmonata. *Zoologica Scripta*, 17 : 239-252.
- WALDÉN, H.W., 1976. A nomenclatural list of the land Mollusca of the British Isles. *Journal of Conchology*, 29 : 21-25.
- WALDÉN, H.W., 1983. Systematic and biogeographical studies of the terrestrial Gastropoda of Madeira. With an annotated checklist. *Annales zoologici Fennici*, 20 : 255-275.
- WEBB, G.R., 1950. Comparative study of mating in two species of arionid mollusks. *Journal of Entomology and Zoology*, 42 : 28-37.
- WEBB, G.R., 1961. The phylogeny of American land snails with emphasis on the Polygyridae, Arionidae and Ammonitellidae. *Gastropodia*, 1 : 31-44.
- WHITE, M.J.D., 1973. Animal cytology and evolution (3rd ed.). University Press, Cambridge, 961 pp.
- WIKTOR, A., 1973. Die Nacktschnecken Polens (Arionidae, Milacidae, Limacidae) (Gastropoda, Stylommatophora). *Monographiae Fauny Polski*, 1 : 1-182.
- WIKTOR, A., 1987a. Spermatophores in Milacidae and their significance for classification (Gastropoda, Pulmonata). *Malakologische Abhandlungen, Dresden*, 12 : 85-100.
- WIKTOR, A., 1987b. Milacidae (Gastropoda, Pulmonata) - Systematic monograph. *Annales Zoologici*, 41 : 154-319.
- WILLIAMSON, M., 1959. Studies on the color and genetics of the black slug. *Proceedings of the Royal Physical Society of Edinburgh*, 27 : 87-93.
- WOTTON, F.W., 1893. The life-history of *Arion ater* and its power of self-fertilisation. *Journal of Conchology*, 7 : 158-167.
- ZEISSLER, H., 1981. Mollusken aus einer Hangwäldchen rechts der Kieler Bucht und einige Bemerkungen zu *Arion owenii* DAVIES, 1979. *Malakologische Abhandlungen, Dresden*, 7 : 137-141.

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Appendix 1

Alphabetical list of the arionid species- and genus-group names mentioned in the text.

1. Genus-group names :

Anadenus HEYNEMANN, 1863
Ariolimax MÖRCH, 1859
Arion FÉRUSSAC, 1819
Ariopelta SIRGEL, 1985
Ariostialis SIRGEL, 1985
Ariunculus LESSONA, 1881
Baudonia MABILLE, 1868
Binneya COOPER, 1863
Carinarion HESSE, 1926
Carinella MABILLE, 1870
Diatriidae SIMROTH, 1885 (a)
Geomalacus ALLMAN, 1843
Hemphillia BLAND & BINNEY, 1872
Kobeltia SEIBERT, 1873
Letourneuxia BOURGUIGNAT, 1866
Lochea MOQUIN-TANDON, 1855
Majorium TORRES MINGUEZ, 1925 (sic)
Mediocrum TORRES MINGUEZ, 1925 (sic)
Mesarion HESSE, 1926
Microarion HESSE, 1926
Monoatriidae SIMROTH, 1885 (a)
Parvorum TORRES MINGUEZ, 1925 (sic)
Prolepis MOQUIN-TANDON, 1855
pseudocarينات TORRES MINGUEZ, 1925 (sic)
subcylindricus TORRES MINGUEZ, 1925 (sic)

(a) SIMROTH (1885a) used family-group names in a (sub)generic sense. These names are not in accordance with Art. 11f of the ICZN.

2. Species-group names with original generic allocation :

alpinus POLLONERA, 1887 (*Arion*)
anguloi MARTÍN & GÓMEZ, 1988 (*Arion*)
ater LINNAEUS, 1758 (*Limax*)
bourguignati MABILLE, 1868 (*Arion*)
brunneus LEHMANN, 1862 (*Arion*)
circumscriptus JOHNSTON, 1828 (*Arion*)
distinctus MABILLE, 1868 (*Arion*)
empiricorum FÉRUSSAC, 1819 (*Arion*)
fagophilus DE WINTER, 1986 (*Arion*)
fasciatus NILSSON, 1823 (*Limax*)
flagellus COLLINGE, 1893 (*Arion*)
franciscocoli BOATO, BODON & GIUSTI, 1983 (*Arion*)
hortensis FÉRUSSAC, 1819 (*Arion*)
intermedius NORMAND, 1852 (*Arion*)
lusitanicus MABILLE, 1868 (*Arion*)
maculosus ALLMAN, 1843 (*Geomalacus*)
magnus TORRES MINGUEZ, 1923 (*Arion*)
minimus SIMROTH, 1885 (*Arion*)
montana MABILLE, 1868 (*Baudonia*)
owenii DAVIES, 1979 (*Arion*)
pegorarii LESSONA & POLLONERA, 1882 (*Arion*)
rufus LINNAEUS, 1758 (*Limax*)
sibiricus SIMROTH, 1901 (*Arion*)
silvaticus LOHMANDER, 1937 (*Arion*)
simrothi KÜNKEL in GEYER, 1909 (*Arion*)
subcarinatus POLLONERA, 1885 (*Arion*)
subfuscus DRAPARNAUD, 1805 (*Limax*)
timidus MORELET, 1845 (*Arion*)
urbiae DE WINTER, 1986 (*Arion*)

Appendix 2

Diagnoses of species-groups in the genus *Arion* as proposed by POLLONERA (1890) :

I. – Groupe de l'*A. empiricorum*.

Animal habituellement grand et unicolore à l'état adulte, rarement orné de bandes; très rarement de petite taille. Bouclier assez grand. Oviducte plus ou moins renflé à sa termination, rétracteur situé au-dessus du renflement se réunissant à celui qui part du canal de la poche copulatrice. Dent centrale avec *aculei*, champs médians avec 2; dans les champs latéraux le petit *aculeus* (externe) se perd, et on ne le retrouve que dans les dernières séries marginales.

II. – Groupe de l'*A. subfuscus*.

Animal généralement de taille moyenne, très souvent orné de bandes. Bouclier assez grand. Oviducte sans renflement terminal bien marqué; rétracteur attaché directement à la poche copulatrice, et étroitement uni à celui de l'oviducte. Radula. Dents des champs latéraux avec le petit *aculeus* (externe) fondu avec le grand *aculeus*, mais toujours visible.

III. – Groupe de l'*A. hortensis*.

Animal généralement de taille assez petite; presque toujours orné de bandes. Bouclier souvent assez petit. Oviducte le plus souvent sans renflement terminal distinct; rétracteur partant du canal de la poche copulatrice se réunissant avec celui de l'oviducte, mais moins étroitement que dans le groupe précédent. Champs latéraux de la Radula avec les deux *aculei* distincts.

IV. – Groupe de l'*A. Bourguignati*.

Différent du précédent pour avoir le dos caréné par la série médiane des tubercules dorsaux plus relevée que les autres; dans tous les autres caractères tout-à-fait semblable au groupe précédent. Mabile (Ann. Malac., 1870, p. 106) donne à ce groupe le nom de *Carinella*.

Appendix 3

Diagnoses of species-groups in the genus *Arion* as proposed by COLLINGE (1897):

1. The *Arion ater* group.

The animal is large and unicolour in the adult. Rarely banded or striped, excepting in young specimens. Mantle large. Adult never keeled. Free-oviduct of moderate length, with its terminal portion usually globose or expanded. Sperm-duct usually, but not always, opens into the vestibule by a thickened ring-like swelling. Retractor muscle of the free-oviduct always situated above the lower portion, and joins with that supplying the receptaculum seminis.

2. The *Arion subfuscus* group.

Usually banded, variable in colour. Varies in size from 35 to 75 millim. Mantle rather large. Adult never keeled. Free-oviduct generally sac-like or wide and long, without well-defined terminal swelling. Duct of receptaculum seminis usually long. Retractor muscles of the free-oviduct and receptaculum seminis usually distinct or only slenderly united.

3. The *Arion hortensis* group.

Nearly always banded, of dark colour. Varies in size from 30 to 55 millim. Mantle medium-sized, often small. Adult never keeled. Sperm-duct usually long and tapering, passing imperceptibly into the vas deferens. Free-oviduct gradually tapering. Retractor muscles usually united, but only slenderly.

4. The *Arion fasciatus* group.

Nearly always banded. In external appearance agree very closely with the preceding group. Body sometimes keeled. Head of receptaculum seminis elongated, duct short.

5. The *Arion intermedius* group.

Animal usually of small size, varying from 15 to 27 millim. Mantle almost circular. Adult never keeled. Receptacular duct short and broad.

Appendix 4

Diagnoses of species-groups in the (sub)genus *Arion* as proposed by Hesse (1926):

Sect. 1. *Lochea* M.-Td., 1855 (ex rect. mea).

Typ. *A. empiricorum* Fér.

Lochea MOQUIN-TANDON, Hist. Moll. France II, S. 10.

Tier groß, Länge über 7 cm, erreicht bis 25 cm; erwachsen stets einfarbig, in der Jugend zuweilen getreift. Schleim gewöhnlich farblos, zähe. Mantelbinden fehlen, im Jugendzustande sind sie mitunter vorhanden, und dann leierartig geschweift. Genitalien: Atrium oft durch eine quere Einschnürung in zwei Abschnitte geteilt. Der obere hat nicht selten eine sackartige seitliche Ausbuchtung und enthält ein eigentümliches konisch aufgerolltes Organ, die Ligula, das am vorderen Ende des freien Oviducts befestigt ist. Der am Oviduct angeheftete breite Arm des Genitalretractors umfaßt teilweise den Oviduct scheidenartig von der Anhaftungsstelle nach dem Atrium zu. Spermatophor mit einer sägezahnartig gezackten Längsleiste und dünnem Schwanzende.

Sect. 2. *Mesarion* n. sect.

Typ. *A. subfuscus* Drap.

Tier von mittlerer Größe, ausgestreckt 35-75 mm lang. Gewöhnlich mit breiten dunkeln Längsbinden auf jeder Seite des Mantels; in der rechten liegt die Atemöffnung. Die Färbung variiert von mattgrau bis gelb und dunkel rötlichbraun. Ohne helle Rückenlinie. Schleim dunkelgelb. Sohle gelblichweiß. Genitalien: Der freie Oviduct relativ kurz, nach vorn breiter werdend. Bursa rundlich. Atrium wesentlich kürzer, als bei *A. circumscriptus*.

Sect. 3. *Carinarion* n. nom.

Typ. *A. circumscriptus* JOHNSTON

Carinella MABILLE, Ann. Malac. 1870, S. 106, nec *Carinella* SOWERBY, 1839.

Farbe gelbgrau oder bräunlichgrau. Die mittlere Runzelreihe des Rückens durch hellere Färbung ausgezogen und dadurch stärker hervortretend, besonders deutlich bei jungen Tieren. Schleim nie dunkelgelb. Sohle rein weiß. Die rechte Mantelbinde verläuft oberhalb der Atemöffnung. Genitalien: Freier Oviduct relativ kurz. Bursa fast immer mit einem spitzen Zipfel. Atrium bandförmig, mindestens doppelt so lang als breit.

Sect. 4. *Kobeltia* SEIBERT, 1873.

Typ. *A. hortensis* Fér.

Kobeltia SEIBERT, Nachr. Bl. V., 1873, S. 81.

Tier Mittelgroß, 30-55 mm lang. Sohle auffallend gelb. Farbe bläulichgrau bei jungen oder bräunlich bei erwachsenen Individuen. Runzeln normal flach. Mantelbinde schwarz, verläuft am Rande des Mantels, oft mit einem schmalen hellen Streifen an der Aussenseite. Genitalien: Der freie Oviduct sehr lang, so lang wie Vas deferens und Epiphallus zusammen; seine hintere Hälfte schlank, die vordere konisch verbreitert. Spermatophor glatt, nicht sägeartig gezackt.

Sect. 5. *Microarion* n. sect.

Typ. *A. intermedius* NORMAND

Tier klein, Länge 15-27 mm, weiß bis gelblichgrau. Ueber dem vorderen Teile des Fußsaums gewöhnlich eine horizontale Reihe dunkler Punkte. Beim zusammengezogenen Tiere haben die Runzeln das Aussehen kleiner Spitzen oder konischer Knötchen. Mantelbinden fehlen meist oder sind undeutlich und verschwommen, in der Form denen von *A. subfuscus* ähnlich; selten deutlich dunkel rotbraun. Genitalien: Freier Oviduct relativ kurz, von gleichmäßiger Breite. Bursa rundlich. Atrium kürzer als bei *A. circumscriptus*. Spermatophor noch nicht beobachtet.

PLATE 1.

SEM photographs of spermatophores of *Carinarion* sp. from Sweden. - A : *A. silvaticus* (Overgrans). - B : *A. silvaticus* (Tierps). - C : detail of the serrated ridge in B. - D : detail of the serrated ridge in A.

PLATE 2.

SEM photographs of spermatophores of *Carinarion* spp. - A : *A. silvaticus* (Vielsalm, Belgium). - B : detail of the serrated ridge in A. - C : *A. fasciatus* s.s. (Teplice, Czechoslovakia). - D : detail of the serrated ridge in C.

PLATE 3.

SEM photographs of a spermatophore of *Arion* (*Mesarion*) *subfuscus* s.l. from Bellevaux, Belgium. - A : complete spermatophore. - B-C : details of the serrated ridge. Remark the variation in dentition between the apical (B) and distal regions (C).

PLATE 4.

SEM photographs of a spermatophore of *Arion* (*Mesarion*) *subfuscus* s.l. from Rouge Cloître, Belgium. - A : complete spermatophore. - B : part of the serrated ridge with transition between different dentition types. - C : apical region where the ridge is continuous and not serrated. - D : detail of the serrations in the upper part of B.

PLATE 5.

SEM photograph of spermatophores of *Arion* (*Mesarion*) *lusitanicus* s.l. from Belgium. - A : apical side of a specimen from Wezembeek-Oppeem. - B : complete specimen from Ste. Cécile-sur-Semois. - C : detail of the serrated ridge in A. - D : detail of the serrated ridge in B.

