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The chromosomes of *Succinea (Succinella) oblonga* Draparnaud, 1801, with considerations on the status of the subgenus *Succinella* Mabille, 1870 (Gastropoda, Euthyneura: Succineoidea)

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ABSTRACT

The cytological conditions in the land snail *Succinea (Succinella) oblonga* are described (n = 12) and compared to those in the genus *Catinella* (n = 6). The mutual relations between the karyotypes of *Succinella* and *Catinella* are discussed. Though the chromosome number of *Succinella* is an exact duplicate of most of the *Catinella* species *Succinella* is not considered a polyploid of one of those. The status of the subgenus *Succinella* is reconsidered, and the recognition of a full generic rank is proposed.

INTRODUCTION AND ACKNOWLEDGEMENTS

The land snail, *Succinea (Succinella) oblonga* Draparnaud, 1801, lives in humid grasslands, in light forests, on overgrown walls and other, often dry places. Its ecological amplitude is relatively great and is coupled with rather broad morphological variation, which resulted in the description of a number of nominal species, the taxonomic status of which needs reconsideration. The species has a European — West Asiatic distribution and occurs in the Alps up to an altitude of 2200 m above sea level.

The chromosome number was reported by Butot & Kiauta (1964). In the present note an account of the chromosome morphology is given. Special attention has been payed to the evolution of the karyotype.

The snails on which this study is based were collected in the Netherlands at Castricum (Prov. of Noord Holland), September 27th, 1964; at Waarder (Prov. of Zuid Holland), May 17th, 1965; on the island of Vlieland (Prov. of Friesland), August 26th, 1965; and at Vierlingsbeek (Prov. of Noord Brabant), June 2nd, 1967. Snails had also been collected from the surroundings

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of Tübingen (Western Germany), May 1st, 1967; at De Panne (Belgium), October 14th, 1964, and at Wieze (Belgium), April 21st, 1966. The snails from De Panne and Waarder did not show good mitotic activity.

In all, 15 specimens were examined and 71 microphotographs were taken. For the methods applied and the equipment used is referred to Butot & Kiauta (1966, 1967).

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CHROMOSOME MORPHOLOGY

The diploid chromosome number in spermatogonial mitosis of *Succinea (Succinella) oblonga* is 24. The following account of the morphological features of the karyotype is based on the karyograms reproduced in pl. I (figs. 1—3).

According to their size, the mitotic chromosomes fall into two groups. The first ten pairs are of slowly decreasing magnitude (6.6—3.5 μ), the 11th and the 12th pairs are considerably smaller and measure about 2 μ. The difference in length between some pairs is often so minute (or even nil) that they could be distinguished by the variation in the position of the centromere only.

The chromosome lengths and the position of the centromere are given in table I. Two pairs are metacentric, four submetacentric, five are subacrocentric and in one pair the position of the centromere could not be determined.

TABLE I. The position of the centromere and chromosome length in *Succinella oblonga*

pair	position	length (μ)
1	median	6.6
2	subterminal	6.6
3	subterminal	5.3
4	submedian	5.3
5	subterminal	5.3
6	submedian	4.8
7	submedian	4.8
8	subterminal	4.0
9	subterminal	4.0
10	submedian	3.8
11	median	2.0
12	?	1.8

At diakinesis 12 elements could easily be observed (pl. II figs. 4—7). At least eight of them have two chiasmata per bivalent (pl. II fig. 6), whereas in at least the smallest two bivalents, only a single chiasma occurs. The number of chiasmata in two bivalents is not certain.

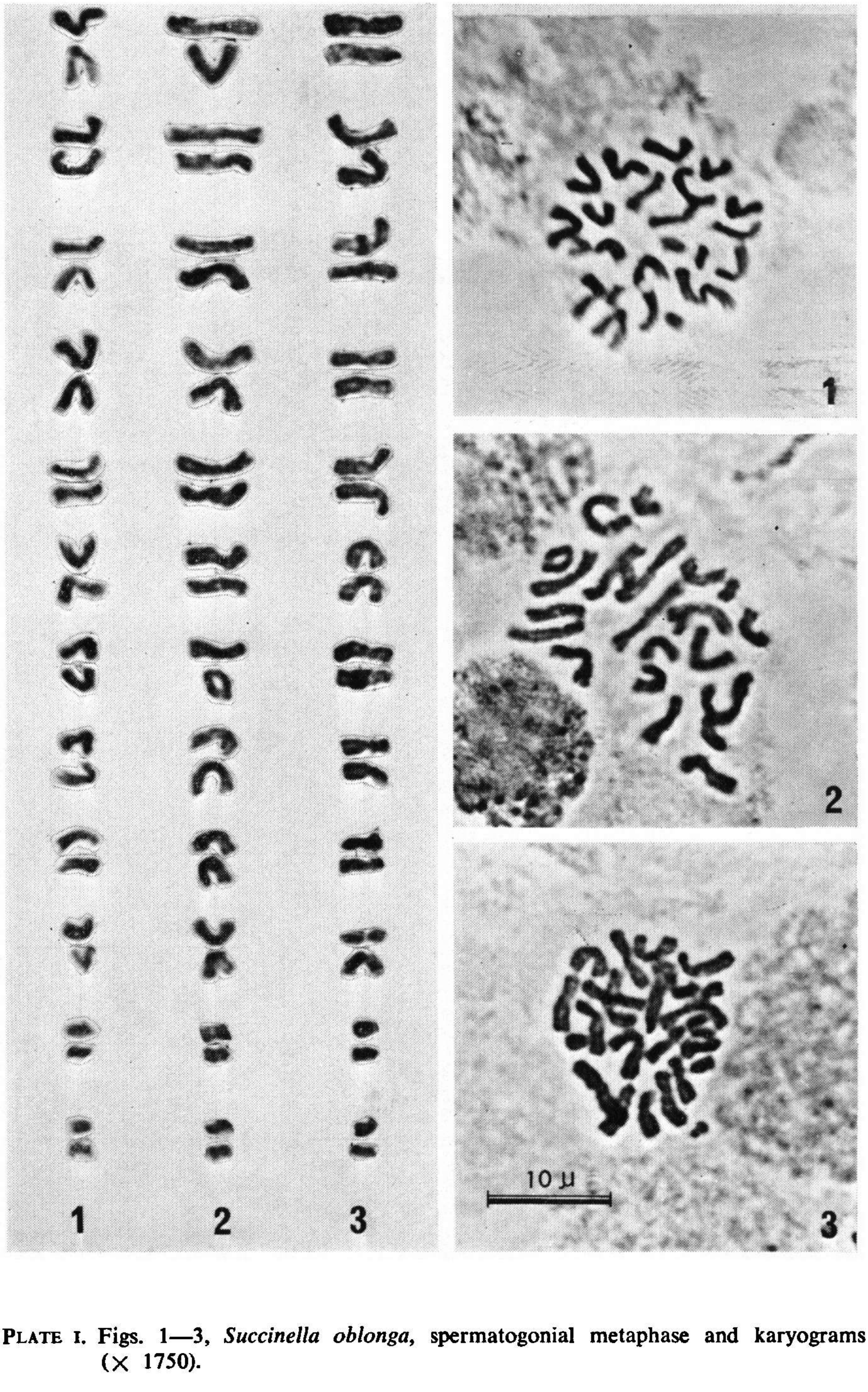


PLATE I. Figs. 1—3, *Succinella oblonga*, spermatogonial metaphase and karyograms (× 1750).

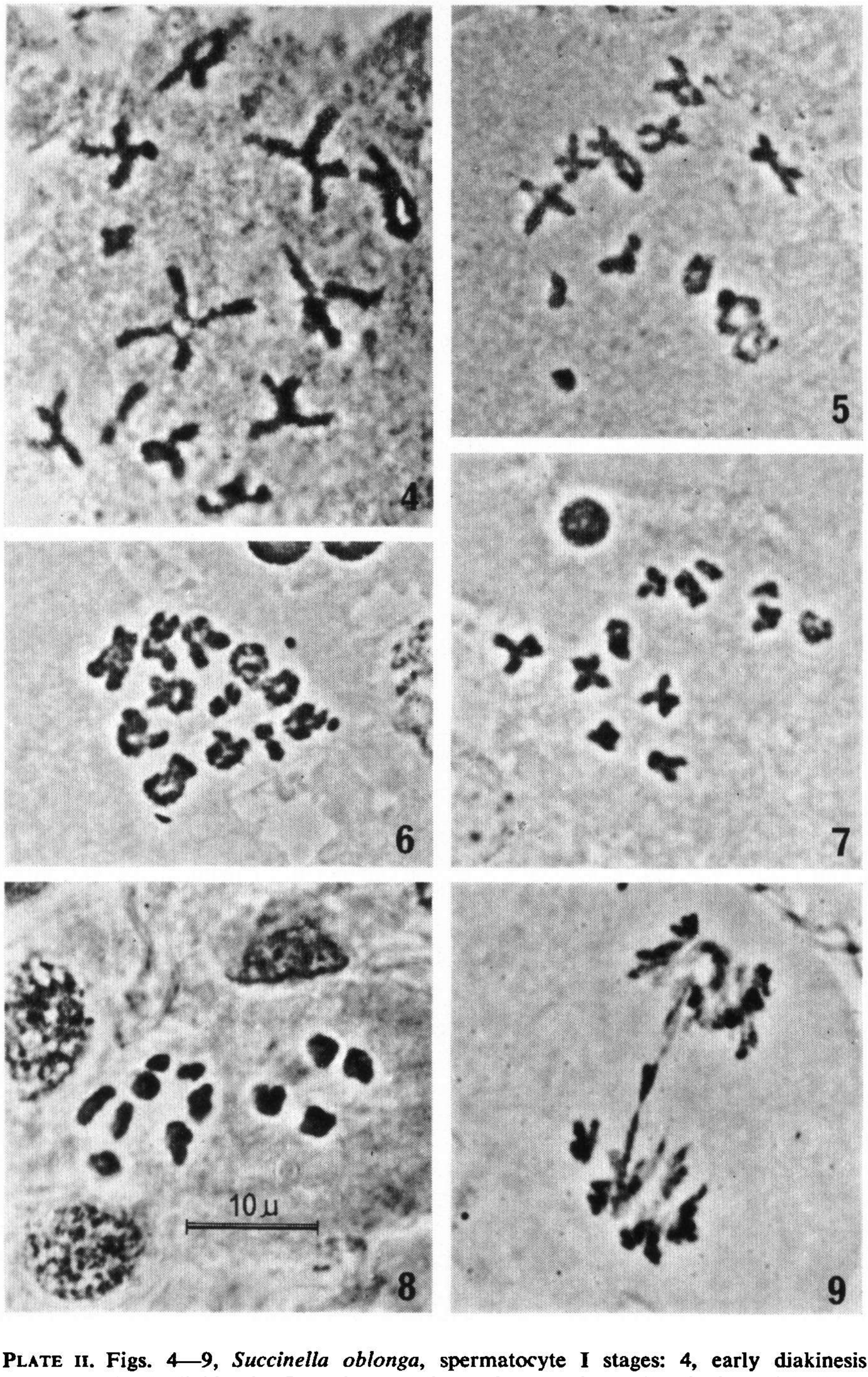


PLATE II. Figs. 4—9, *Succinella oblonga*, spermatocyte I stages: 4, early diakinesis; 5—6, diakinesis; 7, early metaphase; 8, metaphase; 9, telophase (× 1750).

At metaphase I the two small bivalents could easily be distinguished from the other elements (pl. II fig. 8).

At telophase I a bridge was often observed (pl. II fig. 9).

POSSIBLE PHYLETIC RELATIONS BETWEEN THE *Catinella*- AND *Succinella*-KARYOTYPES

The chromosome number of *Succinella oblonga* (n = 12) is unique among the members of the order Succineoidea so far examined. The other haploid chromosome numbers hitherto reported for Succineoidea are 5, 6, 15, 17, 18, 19, 20, 21, and 22 (Burch, Patterson & Natarajan, 1966, and Burch, 1965). The existance of a gap between the low-n (5—6) and high-n (15—22) series is obvious. It is filled up only in part by *Succinella oblonga.* The chromosome number of this snail is an exact duplicate with regards to five of the six *Catinella* species studied; it is 5 in one of them (Burch, 1964). This situation induced Burch, Patterson & Natarajan (1966) and Natarajan, Hubricht & Burch (1966) to suggest polyploidy for *S. oblonga.* Automatically the question rises whether or not *Succinella oblonga* is an euploid (tetraploid) of the complement found in the catinella's. Another alternative for a mutual relation between the *Catinella* and the *Succinella* karyotypes is, of course, the theoretical possibility of fusions of the elements of the n = 12 complement, resulting in a catinelloid complement n = 6. The two alternatives will be briefly discussed in the following paragraphs.

I. Fusion of the elements

The possibility of fusion could cytologically not be excluded. Translocations or the centric fusion of a Robertsonian type, which result in a reduced chromosome number, have played an especially important role in the karyotype evolution of various animals. Tandem fusion and elimination of single chromosomes also result in a reduced chromosome number, but are not considered to have any evolutionary importance (White, 1957). This is due to the disturbances in the meiosis and the lethality or sublethality which they cause (Gustavsson & Sundt, 1967).

The centric fusion is a type of translocation involving the entire arms of rod chromosomes in which two acrocentrics give rise to a large metacentric chromosome and a minute element, which consists for the greater part of heterochromatine and is considered to disappear from the population after a few generations. Recent investigations have revealed also the existence of real fusions of terminally located centromeres (Neelson-Rees, Kniazeff & Darby, 1964), nevertheless, the classical mode of centric fusion remains valid for most animal groups. It leads to a decrease in the number of centromeres in the complement (with loss of the minute element) and is often an important factor reforming the karyotypes. It is peculiar to the animal kingdom, where it is known in insects (Orthoptera, Diptera) and in reptiles.

In the *Succinella-Catinella* it is, in our opinion, not feasible to presume any kind of chromosome fusion. The grounds for such a conclusion are more of phylogenetical than of cytological nature, although with regard to the latter too, it should be stressed, that Patterson (1967) considered "fusion" a very scarce phenomenon in molluscs. Indeed, to our knowledge, not a single reliable case of any kind of chromosome fusion in a mollusc has ever been reported. Moreover, the suggestion of a karyotype evolution in the direction from *Succinella* to *Catinella* is not tenable. It would imply the supposition of phyletic primitivity of the former with respect to the latter, whereas, on anatomical grounds, *Catinella* is generally accepted as the more primitive (Odhner, 1950). This being so, the connection between the *Catinella* and the *Succinella* complements, if any, should be looked for in the sphere of possible polyploidy of the latter which would at least be in agreement with the phylogenetic position of the two groups as it is understood at present.

II. The problem of polyploidy

It is extraordinary difficult to prove whether or not a species is a polyploid of some other species.

In a recent paper Levan & Müntzing (1963) gave a review of the terminology of chromosome numbers. The term polyploid was fixed as the general designation for multiples of the basic number (= haploid = monoploid) higher than diploid.

The term polyploid always implies a mutual phylogenetic relation between two species. Whether or not the chromosome number of the polyploid species is an exact multiple of the monoploid complement is of secondary importance. If, in the course of time, chromosome rearrangements leading to elimination or formation of new elements have taken place in one of the two complements, this original numeric relation will be disqualified.

The term polyploid can only be applied to pairs of species in which an exact euploid descendance can be demonstrated. It is not justified therefore, to suggest a polyploid relation of two species only on the base of comparison of meiotic figures, as has been often done in molluscan cytotaxonomy.

In the case given three criteria were employed and are discussed below.

1. The total length of the chromosomes (TCL).

If *Succinella* were a tetraploid of a *Catinella*, the total DNA content (volume of the chromosomes) of its karyotype should be approximately twice that of the latter. We were unable to determine the chromosome volume, and have had, instead, to rely on the linear measuring of the total length of the mitotic metaphase chromosomes only. It was measured for *Catinella arenaria* in the karyograms published (Butot & Kiauta, 1967) and for *Succinella oblonga* in the karyograms produced in the present paper. The following approximate figures were obtained for the TCL at mitotic metaphase: *C. arenaria* ca. 70, *S. oblonga* ca. 108. We are well aware that the method applied is to a great extent inaccurate since (1) the linear values can only in part give an impression of the approximate volume of the chromosomes, and (2) because a mutual comparison of two seemingly identical figures is hardly justifiable, since the chromosomes in no two figures are really in exactly the same state of contraction. Therefore, the figures obtained by this method alone, are in no way reliable for a final decision as to a possible tetraploidy, though the small difference in length (35%) seems to be in favour of a negative answer.

2. The position of the centromere.

As far as the centromere positions could be determined with certainty, this method gives more realiable results than the one discussed above. If *S. oblonga* were a tetraploid of a catinelloid species, each chromosome of the latter should give rise to a homologous pair of the former. From table II in the paper of Butot & Kiauta (1967) it is evident that *Catinella (Mediappendix) gabbi* (Tryon) and *C. (Mediappendix) vermeta* (Say) lack any acrocentric (or subacrocentric) chromosomes, and, therefore, of all *Catinella* species examined, only *C. (Quickella) arenaria* (Bouch.-Chant.) could be considered here. In table I the positions of the centromeres in *S. oblonga* are tabulated. If the snail was but a tetraploid of *C. arenaria*, four nearly metacentric, four metacentric and four subacrocentric chromosome pairs would be expected in the former. If, on the base of our karyograms, the determination of the centromere positions in *S. oblonga* is correct, the possibility of tetraploidy should be rejected. The more so when the evidence is coupled with the observations discussed in section II, 1. Nevertheless, it should be stressed, in this connection, that if any translocations have taken place in any of the two species discussed, the original centromere positions could secondarily be changed as the result of the translocation processes and then, even in a true tetraploid form, the chromosome morphology is bound to differ from the original diploid form.

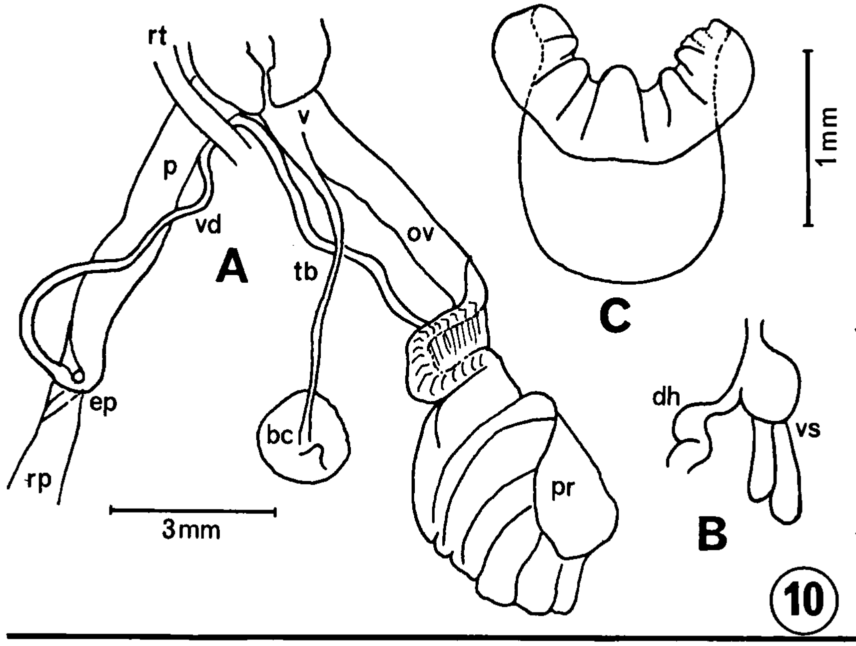
3. The nucleus and cell diameters.

These should be, in a tetraploid species, twice as large as in a diploid form Here again, due to the squashing technique, the evidence is not absolute.

However, it is certain that there is no essential difference between the two species as to this point.

Taking into consideration the above observations, we are inclined to suggest, at least for the time being, that *Succinella oblonga* is not a tetraploid form of any known *Catinella* species. The matter can be finally settled only when more *Catinella* species are examined cytologically, when better karyograms of *Succinella* become available, and when more is known on the principles ruling the evolution of the molluscan chromosome complements.

Rainer (1967) found $n = 11$ and $n = 12$ in different individuals of *Succinella oblonga* belonging to one population living in western Baden (Aargau), Germany. If further research proves the normal haploid number for this species to be $n = 11$, a connection with *Succinea putris* through polyploidy might be possible. This connection is also possible if $n = 12$ turns out to be the normal haploid number. So far, we can only state that the chromosome number $n = 22$ for *S. putris* is an exact duplication of the number found by



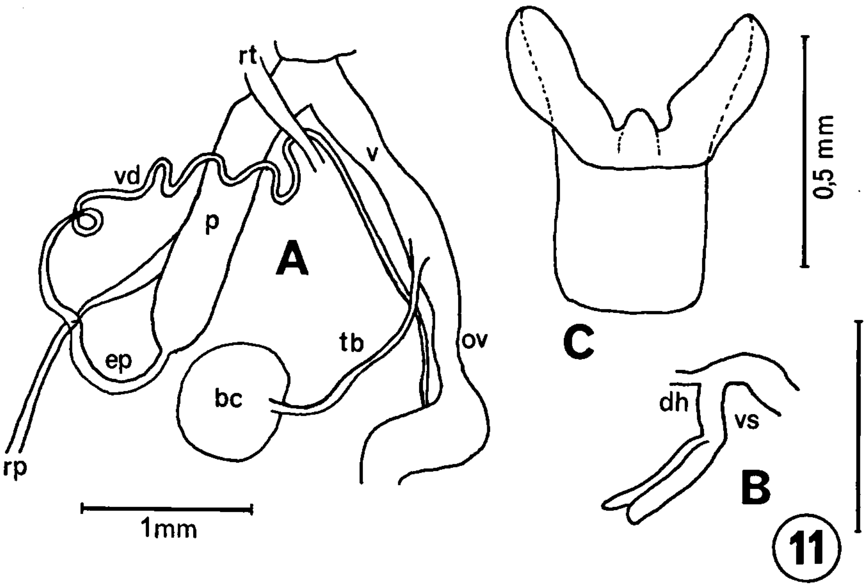


PLATE III. Anatomy of *Succinea putris* (fig. 10) and *Succinella oblonga* (fig. 11): A, reproductive organs; B, vasicula siminalis; C, mandibula. (bc: bursa copulatrix; dh: ductus hermaphroditicus; ep: epiphallus; ov: oviduct; p: penis; pr: prostata; rp: retractor pennis; rt: retractor tentaculi dextri; tb: truncus bursae; v: vagina; vd: vas deferens; vs: vesicula seminalis).

Rainer in one individual of *Succinella oblonga.* Rainer's discovery, be it based on one animal only, is important in that it clearly points to the fact that further research on *Succinella oblonga* is necessary and might solve the problem.

THE TAXONOMIC STATUS OF THE SUBGENUS *Succinella* MABILLE, 1870

So far, the division of the family Succineidae into two subfamilies on anatomical grounds through the possession of a penis sheath (Succineinae) or the absence of it (Catinellinae) is fully supported by their chromosome numbers, which in *Catinella* range from 5—6 and in Succineinae from 12—22.

Although the chromosome number of *Succinella oblonga* is an exact duplicate of that of most of the *Catinella* species, we are of the opinion that *Succinella* has no direct relation with one of the cytologically examined members of the *Catinella* group. It has, no doubt, more affinities in the other subfamily in which it is placed closest to *Succinea putris* Linnaeus and is included in the genus *Succinea* Draparnaud, 1801 of which *S. putris* is the type species.

The genus has been divided into a number of subgroups of which some have been recognized as full genera. The group of which *Succinea oblonga* is the type is known as *Succinella* Mabille, 1870. Thiele (1931) includes the group in his section *Succinea* s.str., though admits that the mandibula is slightly different. Zilch (1959—1960) raises e.g. *Oxyloma* Westerlund, 1885, to generic rank, but regards *Succinella* as "kaum verschieden" and placed it in the genus *Succinea* Draparnaud as a subgenus.

Since *Succinea oblonga* (n = 12) appears very different cytologically from *S. putris*, it is necessary to review other characters which segregate the two species.

The jaw of *S. putris* (pl. III fig. 10 C) shows a strong central projection flanked by another projection at both sides. These three projections are the main ones. One or two minor folds on either side may be observed and appear sometimes as minor projections at the concave edge of the jaw. The jaw of *S. oblonga* (pl. III fig. 11C) is far less solid. It has only the central projection and is comparable to the jaw in a species of the *Oxyloma* group. It cannot be placed in this genus because it lacks an appendix of the penis.

The differences in the radula seem of minor importance as there is a great variability in one and the same species. Single teeth are often abnormal and as often the pattern is asymmetrical. The number of rows varies from 60—95 in the European succineids. For *S. putris* Quick (1933) found 90 (80—94), and for *S. oblonga* 70 (60—75) rows.

The total number of teeth in one row in *S. putris* varies from 59 to 99; in *S. oblonga* the number ranges from 39 to 51. The two *Oxyloma* species in this respect fall within the range of *S. putris*; *S. oblonga* shows numbers closer to *Catinella arenaria* (21—43). The genitalia of *S. putris* and *S. oblonga* (pl. III figs. 10A and 11A, respectively) show similarity only with respect to the epiphallus, the missing appendix and the insertion of the retractor muscle.

The greatest difference is very obvious: the vas deferens is thrown into numerous twists and loops, a feature already mentioned by Quick (1933). No other European species shows these loops which make the vas deferens relatively longer than in *S. putris.* Quick (1933) also mentioned the occurrence of very curious crystalline bodies resembling a hob-nail or a button mushroom, the rounded heads lodging in crypts of the epithelium, the shafts projecting into the lumen of the retracted penis and epiphallus. They are not found in *S. putris.* In *Oxyloma sarsi* Esmark (= *elegans* Quick) the lumen of the penis also contains crystalline bodies; these are, however, of different shape.

The shell needs no descriptive words to distinguish it from *Succinea putris.* It is of very different shape and more liable to be confused with *Catinella arenaria* than with *S. putris.* Conchologically the shell of *S. oblonga* is more primitive than the shell of *S. putris.*

We fully admit that the group of *Succinella* Mabille, 1870 needs comparison with the groups of *Novosuccinea* Pilsbry, 1948; *Calcisuccinea* Pilsbry, 1948; *Desmousuccinea* Webb, 1954, and *Truella* Pease, 1871 which have been classified as subgenera of *Succinea* Draparnaud. Taking into account the above evidence, however, we feel justified to suggest that *Succinella* Mabille, 1870 should be raised to full generic rank in Succineinae.

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