



Naturalis Repository

Biogeography and diversification of the freshwater planarian *Crenobia alpina sensu lato* (Platyhelminthes, Tricladida): A synthetic review

Ronald Sluys

DOI:

<https://doi.org/10.1016/j.jcz.2022.05.010>

Downloaded from

[Naturalis Repository](#)

Article 25fa Dutch Copyright Act (DCA) - End User Rights

This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with consent from the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available following a reasonable period after the work was first published, provided that reference is made to the source of the first publication of the work.

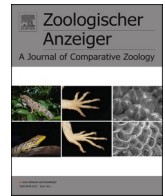
This publication is distributed under the Naturalis Biodiversity Center 'Taverne implementation' programme. In this programme, research output of Naturalis researchers and collection managers that complies with the legal requirements of Article 25fa of the Dutch Copyright Act is distributed online and free of barriers in the Naturalis institutional repository. Research output is distributed six months after its first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and copyrights owner(s) of this work. Any use of the publication other than authorized under this license or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the department of Collection Information know, stating your reasons. In case of a legitimate complaint, Collection Information will make the material inaccessible. Please contact us through email: collectie.informatie@naturalis.nl. We will contact you as soon as possible.

Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Biogeography and diversification of the freshwater planarian *Crenobia alpina* sensu lato (Platyhelminthes, Tricladida): A synthetic review

Ronald Sluys

Naturalis Biodiversity Center, P.O. Box 9517, 2300, RA, Leiden, The Netherlands

ARTICLE INFO

Corresponding Editor: Martin Vinther Sorensen

Keywords:

Crenobia
Distribution
Biogeography
Ice age
Anatomy
Ecology
Karyology
Taxonomy

ABSTRACT

Through an in-depth study of the literature, as well as some other sources of information, geographical distribution maps of the various taxa of the European freshwater planarian genus *Crenobia* (Platyhelminthes, Tricladida) are provided, which document their records and distributional ranges in a detail that was previously unavailable. The genus *Crenobia* ranges across Northern, Western, Southern, and Eastern Europe, including several islands in the Mediterranean region, but is absent from a major part of the Iberian Peninsula; it is distributed also in Turkey. The chromosome portrait of *Crenobia* is complex, as it exhibits chromosome numbers that are multiples of 7, such as 28, 35, 42, 56, and 63. Sexually reproducing populations are tetraploid or hexaploid, while asexually reproducing populations exhibit high chromosome numbers. All taxa of *Crenobia* are stenothermic, cold-loving species. Although historical biogeographic scenarios interpreting the taxonomic diversification of the genus as being the result of the vicissitudes of the Last Ice Age are still captivating, it may be the case that the genus already diversified 6–20 million years ago. The paper sketches in some detail the anatomy of the copulatory complex of the various taxa. Previously, it went unnoticed that in two of the taxa (*Crenobia alpina* and *C. anophthalma*) the two sperm ducts have different, asymmetrical trajectories. Further, it was largely undocumented that in several taxa the sperm ducts exhibit a pronounced recurvature, as well as a dorsal loop before entering the penis papilla. Neither did previous studies document that in *C. montenigrina* the mouth opening is not located at the most posterior end of the pharyngeal pocket, but has shifted anteriorly for some distance. Polypharyngy is a stable, species-specific character, with *C. montenigrina* usually exhibiting 11–17 pharynges, while *C. anophthalma* has never more than three pharynges. Different views have been expressed on the taxonomic status of several geographic and anatomical forms of *Crenobia*. It is shown here that *Crenobia* comprises five, well-defined full species, viz., *C. alpina*, *C. corsica*, *C. montenigrina*, *C. anophthalma*, and *C. bathycola*.

1. Introduction

The planarian species *Crenobia alpina* (Dana, 1766) is a well-known component of the European freshwater fauna, of which the body (up to 16 mm long and 3–5 mm broad) has a rather characteristic external appearance, with its head showing two tentacles and two eyes that are located in unpigmented spots and set quite close together. The tentacles may be developed to greater or lesser extent, depending upon the population; the dorsal colouration is usually greyish or blackish (Fig. 1A and B), excepting two unpigmented forms.

As a result of its distribution, ecological requirements, as well as chromosomal and anatomical characteristics, *C. alpina* sensu lato has always generated much interest from taxonomists, biogeographers, and ecologists. In particular, workers were fascinated by the possibility that

the current distribution and diversification of the genus *Crenobia* Kenk, 1930 may be due to the vicissitudes of the Last Ice Age.

All of this has resulted in a plethora of publications that is unparalleled in any other species of freshwater planarian, or triclad flatworms in general. Steinböck (1942) already remarked that the enumeration of all papers on *Crenobia* would fill several volumes. Although Thienemann (1950), Goubault (1972), and Dahm (1958) published detailed summaries, with particularly the last-mentioned worker contributing also much new information, a comprehensive overview or synthesis was not achieved, due to their different geographical and taxonomic interests and perspectives. The various forms that had been described over the years were variously regarded as good species or as variants of what was called the typical species (“forma typica”, Arndt 1922: 119 ff; Thienemann 1950: 572). Therefore, a clear picture is still missing on the taxonomic

E-mail address: Ronald.Sluys@naturalis.nl.

<https://doi.org/10.1016/j.jcz.2022.05.010>

Received 6 April 2022; Received in revised form 28 May 2022; Accepted 31 May 2022

Available online 3 June 2022

0044-5231/© 2022 Elsevier GmbH. All rights reserved.

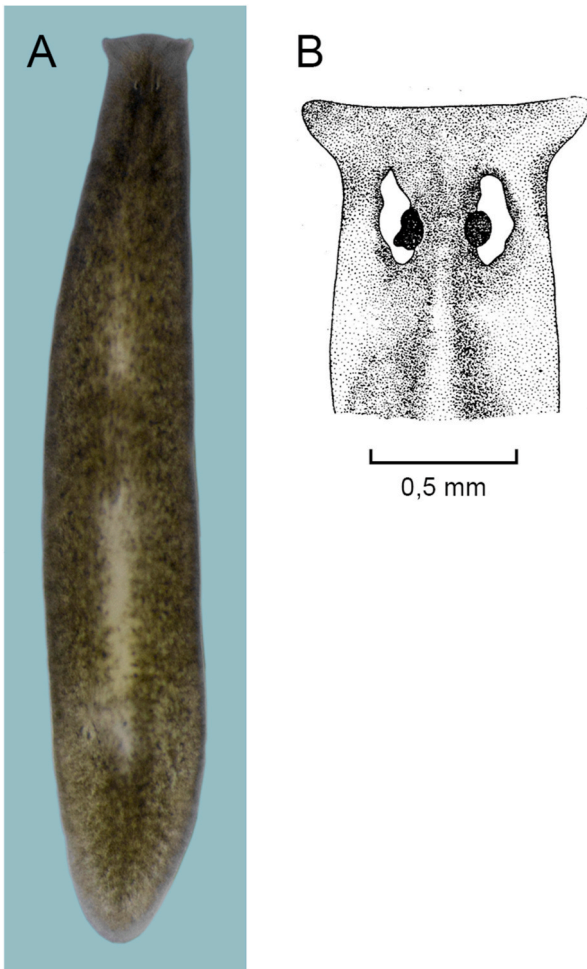


Fig. 1. *Crenobia alpina* (A) External appearance (scale bar unavailable) (B) Detail of head with eyes (after Hauser & Heller 1983).

status and distribution of the various forms, varieties, or subspecies that have been described for *C. alpina* sensu lato.

The present paper aims to remedy this absence of a clear account on the delimitation and evaluation of the various forms of *Crenobia* and their geographic distribution. It does not aim at an exhaustive review of all publications that ever have been written about *C. alpina* sensu lato, but attempts to present a balanced, modern and synthetic treatise, which includes examination of specimens, with an emphasis on the geographic distribution and diversification of the various taxa.

2. Materials and methods

Apart from a large amount of literature, I examined also histological sections of specimens collected from all over Europe. Details concerning the provenance of the specimens studied are given under the appropriate taxonomic headings; all material belongs to the collections of Naturalis Biodiversity Center, Leiden, The Netherlands.

Abbreviations used in the figures: af, atrial fold; bc, bursal canal; cb, copulatory bursa; dlv, dorsal loop vas deferens; ed, ejaculatory duct; fl, fibrous layer; ga, genital atrium; go, gonopore; MfN, Museum für Naturkunde, Berlin; mb, zone of myoblasts; mo, mouth opening; mp, muscle plates; NBC, Naturalis Biodiversity Center, Leiden; od, oviduct; ph, pharynx; pp, penis papilla; te, testes; ov, ovary; USNM, National Museum of Natural History/Smithsonian Institution, Washington; vd, vas deferens.

3. Taxonomy

The taxonomic and nomenclatural history of *C. alpina* sensu lato is rather complex, but, generally, six variants, forms or subspecies have been recognized on the basis of their morphology, mode of reproduction, habitat, and geographic distribution:

- (1) *Crenobia alpina alpina* (Dana, 1766) [*C. a. meridionalis* Thienemann, 1938 + *C. a. alba* (van Oye, 1935)]: Central Europe, the Alps, and Great Britain; one pharynx; reproduction generally sexual but some populations may reproduce only asexually, others sexually, and others may switch between sexual and asexual reproduction (Dahm 1958). In The Netherlands, Vouïte (1929) found that in a population both sexually mature specimens occurred, as well as animals in the process of fission.
- (2) *Crenobia alpina septentrionalis* (Thienemann, 1938): this form occurs in the northern part of the range, from the level of northern Germany into Scandinavia; it has only one pharynx and the reproduction is asexual through the process of autotomy or fission. The first step in such asexual reproduction is the formation of a constriction at one of the lateral body margins (Fig. 2), as mentioned already by Steinmann (1909).
- (3) *Crenobia alpina bathycola* (Steinmann, 1911): a deep-water form from several lakes in the Alps; monopharyngeal; reproduction: sexual (Schmassmann 1923).
- (4) *Crenobia alpina corsica* (Arndt, 1922): a white form, but with eyes, and with a preference for subterranean waters; monopharyngeal; reproduction probably sexual, as the animals have a fully developed reproductive system (Arndt 1922). It is noteworthy that the typical, pigmented form of *C. alpina* also occurs on Corsica but that it lives in different parts of the stream as compared with *C. a. corsica*. This typical form probably also reproduces sexually as the animals have a copulatory apparatus (Arndt 1922). The co-occurrence of the two forms on Corsica is corroborated by

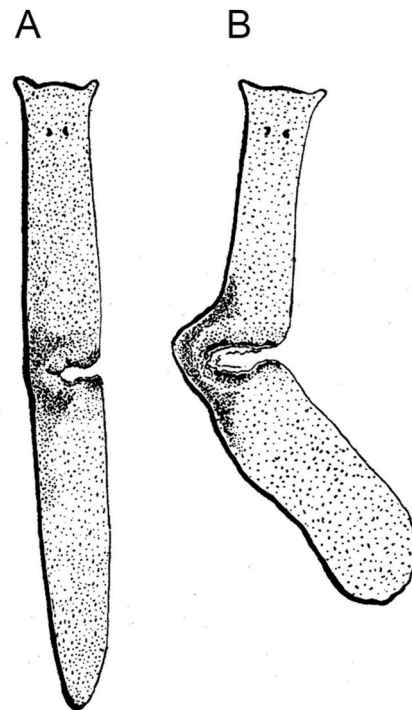


Fig. 2. *Crenobia alpina*. Formation of a constriction at one of the lateral body margins, forming the first step in asexual reproduction through the process of autotomy or fission; A: early stage, B: later stage (after Steinmann & Bresslau 1913).

more recently collected material (see below), with the specimen of the nominate form indeed having developed a reproductive system.

- (5) *Crenobia alpina montenigrina* (Mrázek, 1904) [+ *C. a. teratophilia* (Steinmann, 1908)]: Balkan and southern Italy; polypharyngeal [one central pharynx, plus 11–17 lateral ones (Gourbault 1972; Mrázek 1907), or 23–34 in Bulgarian animals (Chichkoff 1903)] (Fig. 3); reproduction: in the field mainly sexual but also by fission (Stella 1957).
- (6) *Crenobia alpina anophthalma* (Mrázek, 1907): this form has a somewhat disjunct distribution as it has been reported from Montenegro (Crna Gora), Bosnia-Herzegovina, and from northern Spain, albeit that the latter record is poorly substantiated by anatomical evidence (cf. Beauchamp 1932; Gourbault 1972); unpigmented and without eyes; polypharyngeal (but only 3 pharynges); reproduction: not documented, probably sexual, as the animals develop a copulatory apparatus (cf. Mrázek 1907).

With respect to the two first-mentioned forms, it is important to note that Thienemann (1938) described the two subspecies *C. a. septentrionalis* and *C. a. meridionalis*. These two races were considered to represent the typical *C. alpina* (“forma typica”, Thienemann 1950: 571). Through his taxonomic act, Thienemann (1938) incorrectly suppressed the name of the nominotypical subspecies, viz., *Crenobia alpina alpina*. Since the type locality of *C. alpina* is in the Alps (Dana 1766) the

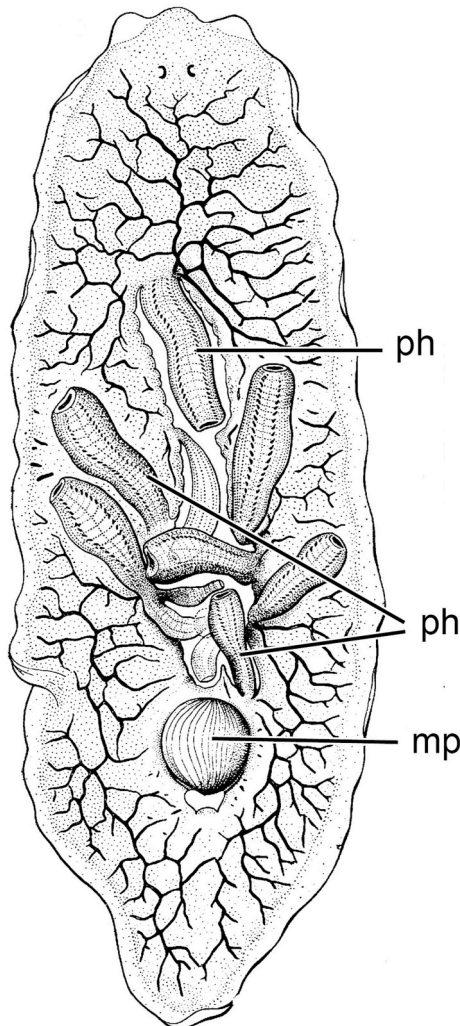


Fig. 3. *Crenobia a. montenigrina*. Preserved specimen showing 10 pharynges and the radial muscle plates around the genital atrium (after Beauchamp 1961).

subspecies *C. a. meridionalis* must be considered as a junior synonym of *C. a. alpina*, as already noted by Kenk (1974) (see also Mayr 1969). This still expresses Thienemann’s (1938, 1950) views that these two forms are simply varieties of *C. alpina*.

In their analysis of mitochondrial DNA and allozymes, Brändle et al. (2007) found evidence for two central European lineages and speculated that these may correspond to the subspecies, if not cryptic species, *meridionalis* and *septentrionalis*. Although it is debatable whether the level of genetic variation reported is sufficient for the recognition of new species, that study also neglected the taxonomic nomenclatural rule on the nominotypical subspecies.

4. Distribution

Only few workers have endeavoured to produce maps in their accounts on the geographic distribution of *Crenobia*. Although Vandel (1922) mentions that *C. alpina* is common in northern Scandinavia, his map is restricted to Central Europe. Despite the fact that the map of Gourbault (1972), here reproduced as Fig. 4, extends to eastern Europe, it equally ignores regions beyond 55° northern latitude and also does not include localities then already known for parts of northeastern Europe, such as Poland and Latvia. Nevertheless, her map is important as she plots many localities, not only of the “typical” form *C. alpina* but also of the taxa *C. a. montenigrina* and *C. a. anophthalma*, which she considers to be separate species.

It remains a matter of speculation why Gourbault (1972) did not incorporate the sampling localities of Dahm (1958), not even the sites that fall within the boundaries of her map. Unfortunately, Dahm (1958) himself did not provide a map, but on the basis of his data I have prepared a distribution map in which his sampling localities (116 in total) are represented (Fig. 5). As Dahm (1958) also detailed whether a particular population reproduced asexually, sexually, or showed both kinds of reproduction, the reproductive modes have been reflected in the plotted data (Fig. 5). Inclusion of these reproductive modalities is one of the differences between Fig. 5 and the map of Lorch et al. (2016), who also plotted Dahm’s localities.

However, even Dahm’s (1958) localities (Fig. 5) and Gourbault’s map (Fig. 4) taken together do not yet represent a comprehensive overview of the biogeographic data potentially available to these workers, apart from information that became available more recently. Fig. 6 presents a third map, including taxa, localities, and/or reproductive modes that are mostly not represented in the data of Dahm (1958) and Gourbault (1972).

In Eastern Europe monopharyngeal and polypharyngeal (*C. a. montenigrina*) forms geographically occur together, a situation that is not reflected at all in Gourbault’s (1972) map. However, here the two forms may be spatially isolated because they inhabit different biotopes. The polypharyngeal form occurs at spring heads and in the reservoirs of rheocene springs and spring-fed streams at a maximum distance of 20 m from the spring. The monopharyngeal form occurs in sections of the streams where the flow of water is higher (Babalean 2017). These recent results contradict the conclusion of Codreanu (1956) that the monopharyngeal and polypharyngeal forms may be found in the same springs and are only separated by a difference in their reproductive period, which takes up the whole year in the former and only autumn, winter and, perhaps, the beginning of summer in the latter.

Although Gourbault’s map (see Fig. 4) depicts three localities in western Turkey, it is not made clear that the eastern-most Turkish record of *Crenobia a. alpina* actually falls outside the boundaries of her map. And neither is it obvious that there was already a record of *C. a. montenigrina* on Crete. The records for Turkey and Crete available to me have been represented in Fig. 7.

Analysis of these four maps (Figs. 4–7) learns that *C. alpina* sensu lato, thus including its various forms and subspecies, is distributed in Northern, Western, Southern, and Eastern Europe, and also in Asia Minor, i.e., in Turkey. It is noteworthy that the species does not occur on the major

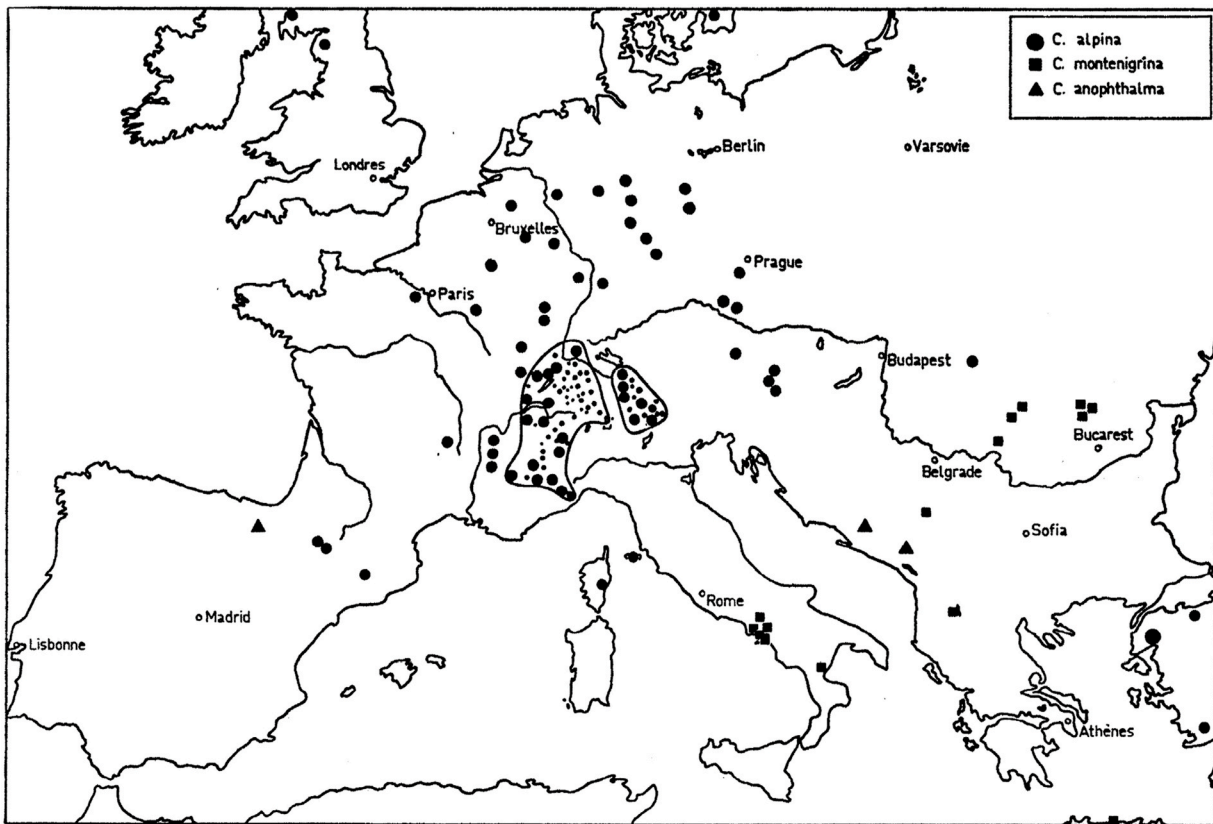


Fig. 4. Geographic distribution of three forms of *Crenobia alpina*, after Gourbault (1972).

part of the Iberian Peninsula, and that it is distributed far in the north of Scandinavia, and is even present on the Faroe Islands.

Where its preferred habitat (see below) is abundantly present, *Crenobia* is also very common, as exemplified by the many sampling localities from which Hoffmann (1964) obtained specimens in Luxembourg and the numerous sites in the Rhön mountains in central Germany (see Rhön 2022).

5. Karyology

The karyology of *Crenobia* is very complex and confusing. The chromosomes are very small and the chromosome numbers are 28, 35, 42, 49, 56, and 63 (i.e., multiples of 7) (Dahm 1958; Benazzi and Benazzi Lentati 1976).

With respect to the chromosomes, it is interesting to note that Dahm (1958) found that strictly sexually reproducing populations showed only tetraploid (28) or hexaploid (42) complements and that the sexual population from The Netherlands studied by van der Linden (1969) was hexaploid. Dutrillaux & Lenicque (1971) reported on a population from the Savoy Alps in France that showed a complement of 42 chromosomes, but unfortunately, did not report on the mode of reproduction of this French population. However, these workers examined several specimens, which, apparently, showed the same chromosome number. Therefore, this population does not fall in the group of populations that shows variable numbers of chromosomes, either among individuals or even within the same specimen, and that are characterized by asexual reproduction. Thus, presumably this French population reproduces sexually. According to Hoffmann (1964), in Luxembourg all populations of *Crenobia* reproduce sexually, while for one population he identified a hexaploid chromosomal complement.

Lorch et al. (2016) analysed the relationship between ploidy level, latitude, and mode of reproduction on the basis of the data for *Crenobia* provided by Dahm (1958). Latitude was considered as a proxy for

environmental conditions, in particular, high latitude implying harsh climatic conditions. A significant positive correlation was found between chromosome numbers and latitude. Their results corroborated the notion that in *Crenobia* asexual individuals are more frequent at higher latitudes (see also Fig. 5) and that there is a relationship between the level of ploidy and mode of reproduction, in that populations with high chromosome numbers generally reproduce asexually by fission, whereas populations with comparatively low numbers, albeit hexaploid, reproduce sexually.

In the case of *Crenobia*, polyploid populations harbor multiple variants of genes that through recombination may effectuate quick adaptation to new environmental conditions and thus facilitate range expansion (Lorch et al. 2016).

6. Ecology

Much has been written about the ecology of *C. alpina*. Generally, it occurs in springs, spring-fed streams and in small, cool, and swiftly flowing streams. This means that it is usually found at places where the water temperature is rather low. So, it has been concluded that *C. alpina* prefers low temperatures and it has been shown that it is unable to tolerate temperatures above 12–14 °C and has a preference of temperatures slightly above 0 °C (see Vila-Farré & Rink 2018 and references therein). On the other hand, *C. alpina* survives in mountain environments with a daily temperature fluctuation of 10–15 °C, with temperature peaks of 25 °C. However, in these cases average temperature throughout the day and night should be about 10 °C (Vila-Farré & Rink 2018 and references therein). Since *C. alpina* can tolerate the higher temperatures in the lower reaches of streams, as well as the lower rate of flow in such stretches, other factors than temperature and stream velocity may influence also its distribution, such as amount of available oxygen (Hauser 1953; Vila-Farré & Rink 2018). All-in-all, it is generally concluded that *C. alpina* sensu lato is a stenothermic, cold-loving species.

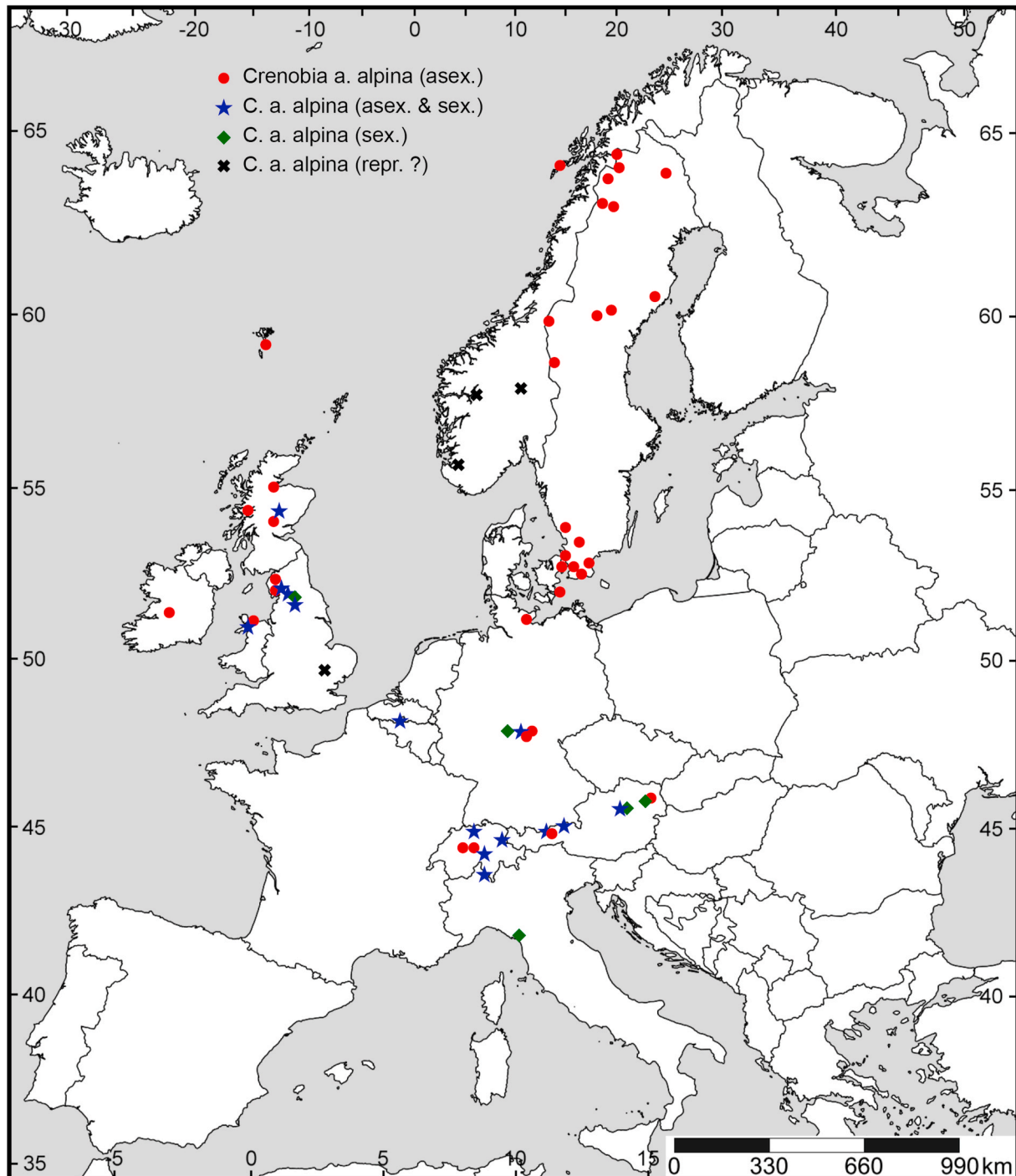


Fig. 5. Sampling localities from [Dahm \(1958\)](#). Localities in close proximity, in relation to the present map, have been pooled. Filled red circle: *C. alpina*, asexually reproducing population(s); filled blue asterisk: *C. alpina*, population with both sexually and asexually reproducing individuals; filled green diamond: *C. alpina*, sexually reproducing population; black cross: *C. alpina*, type of reproduction unknown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Because of its narrow thermal tolerance, combined with its limited dispersal capability, *Crenobia* runs the risk of local extinction when climatic events impinge upon its preferred habitat. This may result in warmer conditions, as well as reduced prey abundance, which negatively affect *Crenobia* in its interspecific exploitation competition with another freshwater planarian, viz., *Phagocata vitta* (Dugès, 1830) ([Durance & Ormerod 2010](#)).

7. Historical biogeography

This preference of *Crenobia* for low temperatures is the reason that in the literature the present-day distribution of *Crenobia* often has been interpreted as having been influenced by the Ice Age (see [Thienemann 1950](#); [Dahm 1958](#) for summaries of the literature). About 18,000 years ago, northern Europe was covered by ice-sheets, which could be 3000 m high ([Osborne & Tarling 1995](#)). The southernmost portion of these ice-sheets covered more or less the northern half of The Netherlands and

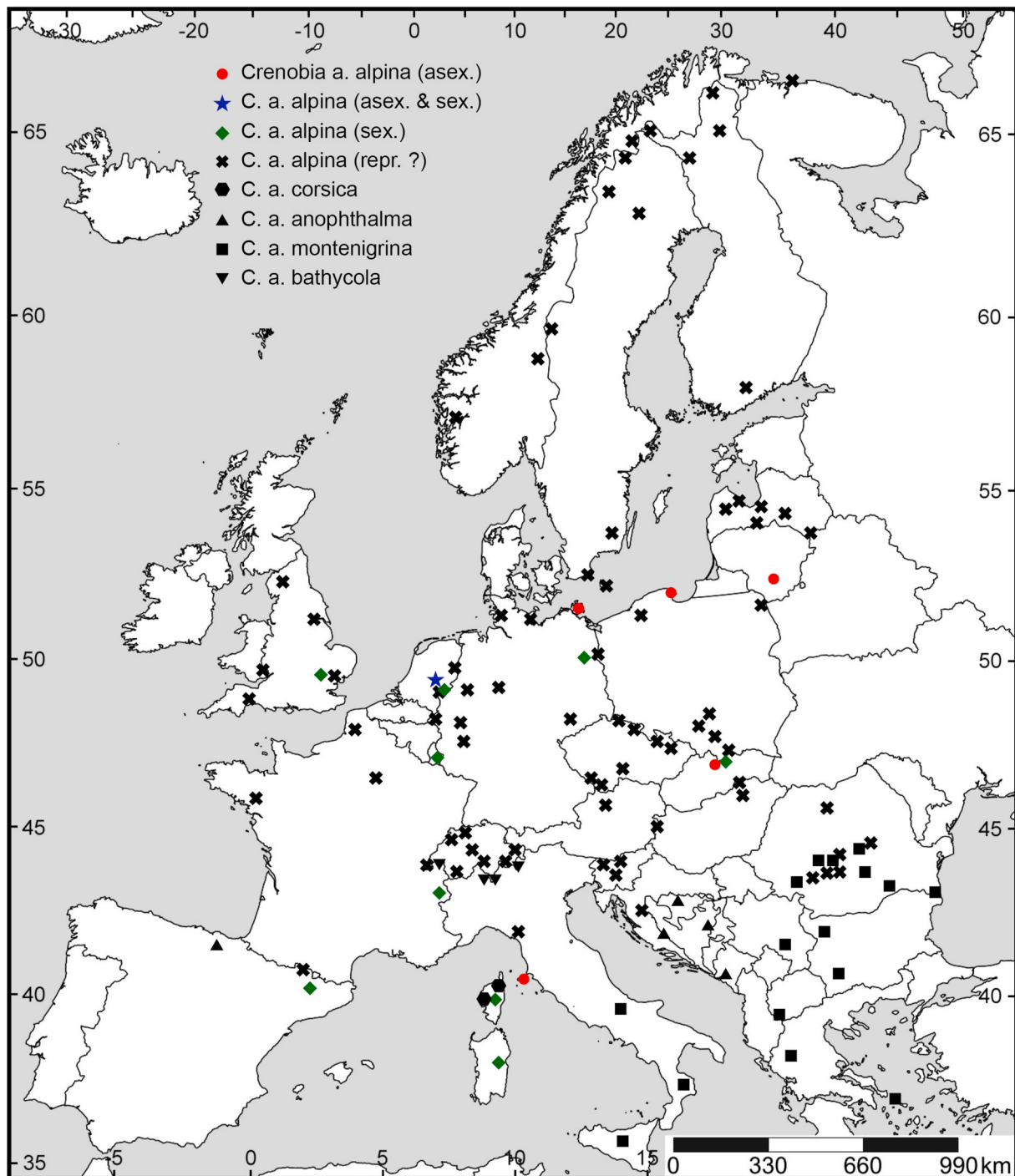


Fig. 6. Sampling localities from: Volz (1901*); Mrázek, 1907; Komárek (1920); Steinmann (1911); Hofsten (1916*, 1919); Roszkowski (1921, 1930*); Schmassmann (1923); Arndt (1922, 1926), Poliński (1926*); Ischreyt (1927); Beauchamp (1932); Pagast & Froese (1933*); Petersen (1935*); Ulyyott (1936); Thienemann (1938); Soós (1943); Benazzi (1946, 1947); Steinböck (1949*); Adlmanseder (1953*); Luther (1961*), Hoffmann (1964*); Oomen & Geelen (1966); Wegelin (1966*); van der Linden (1969); Dutrillaux & Lenicque (1971); Năstăsescu (1974*); Giesen-Hildebrand (1975); Kenk (1978); Pala et al. (1980*); Van der Velde & Cuppen (1981); Witzleb (1989*); Roca et al. (1992*); Geissen (1993); Fülep (2009); Horstmann (2013*); Babalean (2017); Stocchino (2018*); Arrow (2019*); Beracko and Revajová (2019); Okoń et al. (2020); USNM 53404, USNM 58677: Jastrebac, Serbia (*C. a. montenigrina*); USNM: 53400, 53401, 53402, 53403, 58673, 58674, 58675, 58676 (*C. a. alpina*, various localities in Slovenia and Croatia); MfN*: 6365, 7150, 725-Q, 8150, 8153, 8200, 8255, 8584, 9726, 9727 (*C. a. montenigrina*, various localities in Italy and eastern Europe); NBC*: localities of specimens examined for the present paper (see below, under the various taxonomic headings).

Filled red circle: *C. a. alpina*, asexually reproducing population(s); filled blue asterisk: *C. alpina*, population with both sexually and asexually reproducing individuals; filled green diamond: *C. a. alpina*, sexually reproducing population; black cross: *C. a. alpina*, type of reproduction unknown; filled octagonal: *C. a. corsica*; filled triangle: *C. a. anophthalma*; filled black rectangle: *C. a. montenigrina*; inverted (top-down) filled triangle: *C. a. bathycola*.

* Localities in close proximity, in relation to the present map, have been pooled. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

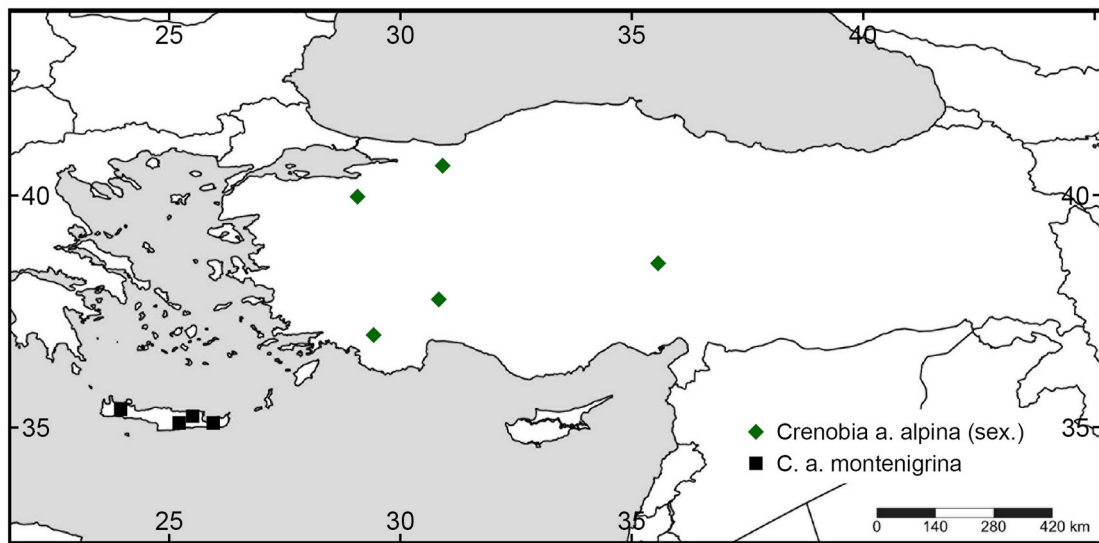


Fig. 7. Distribution of *Crenobia alpina alpina* in Asia Minor (from [Kosswig & Ermin 1950](#)) and *C. a. montenigrina* in Crete (USNM 57873, Meskla; see also [Kenk \(1930\)](#); localities of specimens of *C. a. montenigrina* on Crete examined for the present paper - see below, under Material examined; localities in close proximity, in relation to the present map, have been pooled). Filled green diamond: *C. a. alpina*, sexually reproducing population; filled black rectangle: *C. a. montenigrina*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

covered most of the United Kingdom and Ireland. A smaller glaciation was centred on the Alps and covered parts of Austria, Italy, France, and southern Germany. Evidently, everything that these days lives in these northern parts that were covered with ice 18,000 years ago, must have invaded these parts after the ice had retreated. Therefore, probably the northern form *C. alpina septentrionalis* evolved at the southern border of the ice front and then when the climate changed, it followed the receding glaciers to the north. This invasion or colonization of the northern part was facilitated by the fact that this form reproduces only asexually. In Scandinavia this form is now widespread in the Fennoscandian mountains ([Dahm 1958](#)).

The other form, *C. alpina alpina*, probably occupied a more southern range during the maximum extent of the glaciation, 18,000 years ago. But when the climate changed and it was getting warmer, it could only live in suitable, cold habitats and thus it resorted to spring-heads, etc. There, it generally has a much more sporadic occurrence, apart from the Alps, where there are many suitable locations. It has been suggested that the Alps actually formed the original range of the species and that from thereon the species invaded northern Europe by following the receding ice front ([Steinböck 1942](#)). This Central-European form – *C. alpina alpina* – presently occurs also on Corsica and Sardinia and thus apparently managed to colonize these islands. Due to lowered sea levels during the Ice Age, Corsica and Sardinia were connected with each other and also with the mainland of Italy ([Zunino & Zullini 1995](#): fig. 7.12). It may be that these connections facilitated the dispersal of *Crenobia* to Corsica and Sardinia. In that respect, it is interesting to note that *C. alpina* has been reported also from the small island of Elba (see [Fig. 5](#)), which during the Ice Age formed part of the connection with the mainland of Italy ([Zunino & Zullini 1995](#): fig. 7.12). [Benazzi \(1961\)](#) noted that the conditions during the last glacial age (Würm) would have made it possible for *Crenobia* to reach the island of Elba.

But then we still have to explain (1) the occurrence of another form on Corsica (*C. a. corsica*), (2) presence of the deep-water form *C. a. bathycola* in several lakes in the Alps, (3) the rather wide distribution of the polypharyngeal form *C. a. montenigrina* on the Balkan and southern Italy, (4) and the disjunct distribution of the polypharyngeal form *C. a. anophthalma* (only 3 pharynges) in Montenegro, Croatia, Bosnia-Herzegovina, and northern Spain. I shall not attempt to explain their distributions in the context of the Ice Age, as this would lead us even further into the realm of speculation, if not phantasy.

And there is even a distinct possibility that the Ice Age had no effect whatsoever on the differentiation of the various forms of *Crenobia*. A molecular timetree of *Crenobia* dated the divergence of the major lineages into the Miocene, that is, 6–7 million years ago (Mya) and on the basis of another calculation even to 10–20 Mya ([Brändle et al. 2007](#)). Clearly, these workers could not calibrate their phylogenetic trees on the basis of fossil evidence, as there are no reliable fossils for triclads. They used some measures taken from other studies on other organisms, while their estimates did not have any known margin of error. Nevertheless, they concluded that even despite these uncertainties, the hypothesis can be rejected that *Crenobia* diversified during the Holocene (about 12,000 years ago – recent), i.e., after the last Ice Age.

8. Taxonomic re-evaluation of the various forms

Now that in the above a clear picture has emerged on the distribution of the various subspecies of *C. alpina* sensu lato, it is opportune to re-evaluate the taxonomic status of these forms. This re-evaluation is based on the anatomical analysis of series of specimens from all over Europe and on additional information that recently has become available, notably molecular studies. Furthermore, this re-evaluation takes as its lead the phylogenetic species concept (cf. [Sluys 1991](#); [Sluys & Hazevoet 1999](#)) and the general lineage concept ([de Queiroz 1998, 2007](#)). At first, a short account is presented on some structures of the reproductive system that are characteristic of the genus *Crenobia*. Thereafter, short descriptions are given of the possibly distinguishing anatomical features of the various forms, followed by a taxonomic evaluation of these characters and the implications for the systematic biological status of these taxa.

8.1. The genus *Crenobia*

Detailed accounts on the copulatory apparatus of *Crenobia*, including original sagittal reconstruction drawings, were published by [Kennel \(1888\)](#), [Mrázek \(1904, 1907\)](#), and [Micoletzky \(1907\)](#). Another early account, albeit under the name *Planaria subtentaculata*, was that by [Vejdovsky \(1890\)](#). However, the anatomical examinations of the last-mentioned worker were performed only on a whole specimen under a coverslip and on a horizontal section. Surprisingly, there are no modern studies that evaluated the descriptions of these older workers,

albeit [Beauchamp \(1961\)](#) and [Ball & Reynoldson \(1981\)](#) provided reconstruction drawings of the copulatory complex.

The most conspicuous, if not apomorphic, character of the genus *Crenobia* resides in the extremely thick coat of muscles that surrounds the genital atrium. The major component of this musculature consists of longitudinal fibres that are grouped into crescent-shaped bundles or plates, which are arranged in a radial fashion around the atrium. The thick bands or bundles of longitudinal muscle are immediately obvious in sagittal sections, while their composition and radial arrangement is evident from transverse sections ([Fig. 8](#)).

Interpolated between the muscle plates and the atrial wall, there is a rather thick layer of fibrous tissue, composed of more or less circularly running fibres. The staining properties of this fibrous layer are markedly different from those of the muscle plates. In trichrome stains (e.g., Mallory-Cason/Heidenhain), the muscle bundles generally stain dark red, whereas the fibrous layer stains bright, pale blue ([Fig. 9A](#)). [Mrázek \(1904\)](#) already reported that the staining properties of the fibrous layer are different from the muscles.

On its outer surface, the entire coat of muscles around the genital atrium is surrounded by a rather thick, ball-shaped zone of cells with distinct nuclei ([Fig. 9B](#)). These cells have been variously interpreted as being of a glandular nature or being the myoblasts belonging to the muscle plates, while it has been suggested also that both types of cells are represented in the broad outer zone. I found no evidence for gland cells and, thus, presume that these cells indeed concern myoblasts.

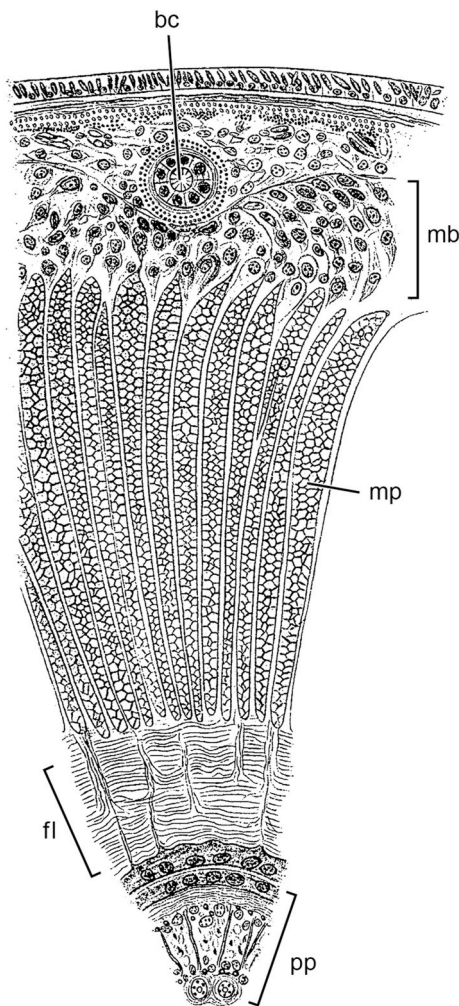


Fig. 8. *Crenobia alpina*. Transverse section through the wall of the genital atrium and the penis papilla, showing the radial muscle plates (after [Micoletzky 1907](#)).

The genital atrium is divided into two regions by a sphincter-like fold in the atrial wall. Immediately anterior to this fold, the dorsal wall of the atrium receives the openings of the bursal canal and the common oviduct. The latter receives the openings of shell glands, which discharge their secretion also into the separate oviducts, well before they have united to form the common oviduct. The bursal canal is a rather narrow duct that communicates with a generally small, rounded or sac-shaped copulatory bursa that is located in the dorsal body region immediately anterior to the male copulatory complex. The penis papilla is a slender, conical structure, with a more or less horizontal orientation; it is only weakly muscularized.

8.2. Taxonomic status

In the literature on *Crenobia*, the polypharyngeal forms *montenigrina* and *anophthalma* have variously been regarded as good species ([Beauchamp 1932](#); [Gourbault 1972](#); [Dahm & Gourbault 1978](#)) or as variants of the single species *C. alpina* ([Kenk 1974, 1978](#)). The following section (a) presents in some detail how the various forms differ taxonomically from each other, (b) determines the taxonomic weight of the characters, and (c) re-evaluates the species or subspecies status of these forms.

8.2.1. *Crenobia a. alpina*

8.2.1.1. Material examined. RMNH VER. 20021.a, Lac Melu, Corsica, France, coll. H. Malicky, sagittal sections on 2 slides of an asexual specimen; ZMA V.Pl. 736.1, 5 km east of Col de Vergio, in Forêt de Valdo-Niello, Corsica, France, 18 June 1981, coll. H. Malicky, sagittal sections on 3 slides; RMNH VER. 21011.a, *ibid.*, sagittal sections on 7 slides; RMNH VER. 21011.b, *ibid.*, sagittal sections on 4 slides; RMNH VER. 21012.a, Ashwell Springs, Hertfordshire, England, 23 May 1975, coll. I. R. Ball, sagittal sections on 3 slides; RMNH VER. 21013.a, near Gavarra, Lleida, Spain, 14 February 2010, coll. M. Vila-Farré & X. Coll, sagittal sections on 3 slides; RMNH VER. 21013.b, *ibid.*, sagittal sections on 2 slides; RMNH VER. 21013.c, *ibid.*, sagittal sections on 3 slides.

8.2.1.2. Comparative description and discussion. The nominate form is provided with a pair of eyes, a single pharynx, a mouth opening that is located at the very posterior end of the pharyngeal pocket, and rather small, prepharyngeal testes. The pharynx is rather large, measuring up to 1/3rd – 1/4th of the body length in preserved specimens, according to [Micoletzky \(1907\)](#) up to 1/4.5. The size of the penis papilla is such that it generally extends posteriad to, or even beyond the atrial constriction.

It seems to have escaped the attention of all previous workers that in fully developed specimens of this form the course of the two vasa deferentia is asymmetrical, with one duct following a trajectory that is considerably different from the other one. One of the sperm ducts, generally expanded to form a spermiducal vesicle, travels up to about the level of the atrial fold and then starts to recurve and to follow a postero-dorsal course, meanwhile bending also towards the median portion of the body. During this trajectory, the sperm duct generally decreases in diameter. Upon reaching a position immediately anterior to the median part of the muscular coat around the atrium, the sperm duct continues its course towards the dorsal body surface for a short distance. Hereafter, it exhibits a kind of loop by recurving in such a way that it penetrates the muscular wall in posterior direction and then communicates with the other vas deferens to form the ejaculatory duct ([Fig. 10](#)).

The other sperm duct follows a completely different course, in that it does not exhibit the extended recurvature, but immediately bends to the dorsal body surface after having reached the anterior level of the muscularized atrium. Directly anterior to the median portion of the ball-shaped coat of cells surrounding the coat of radial muscle plates, this sperm duct shows a similar short loop before penetrating the muscle layer and communicating with the other sperm duct ([Fig. 10](#)).

However, in specimens of *C. a. alpina* one may encounter also a

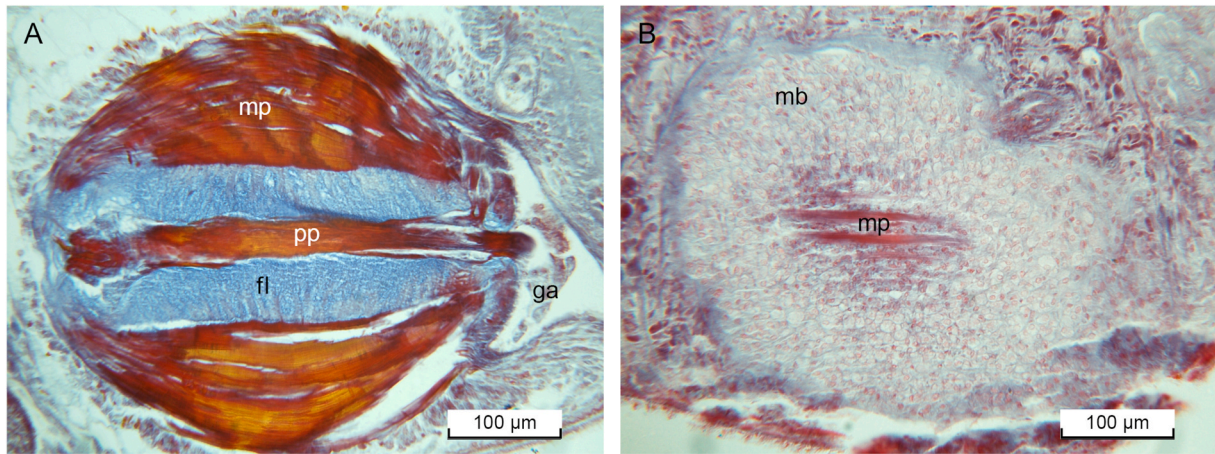


Fig. 9. (A) *Crenobia a. alpina*, RMNH VER. 21013.b, sagittal section, photomicrograph of copulatory apparatus showing the bright blue staining fibrous layer (B) *Crenobia a. montenigrina*, RMNH VER. 21020.c, sagittal section, photomicrograph of zone of myoblasts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

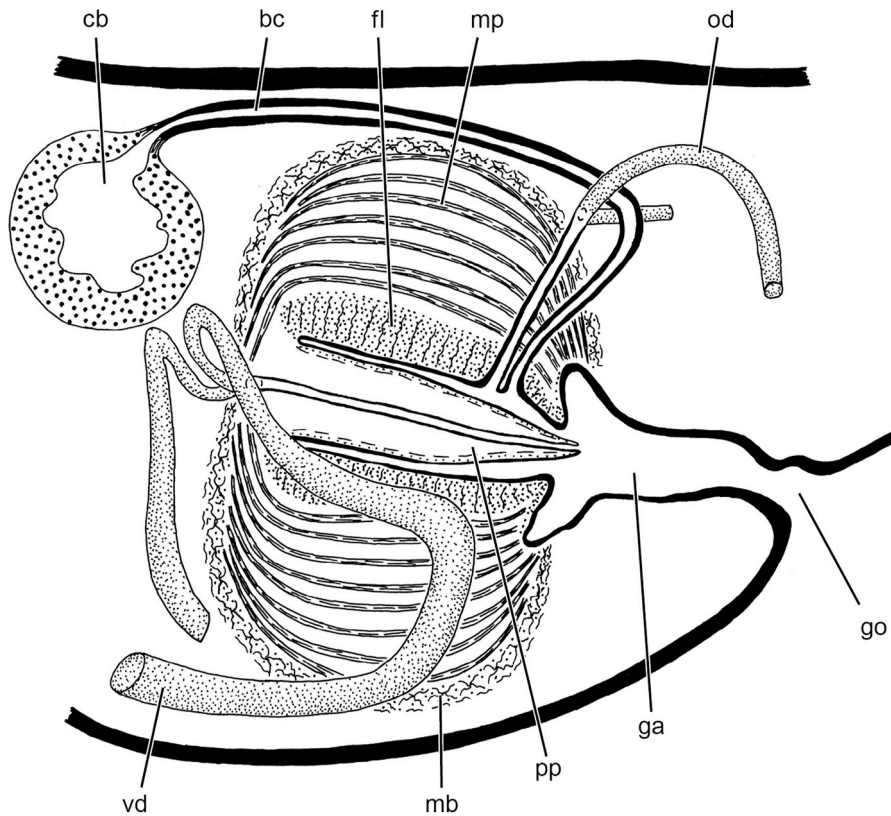


Fig. 10. *Crenobia a. alpina*. Diagrammatic reconstruction of the copulatory apparatus.

condition in which the sperm ducts exhibit symmetrical trajectories, in which both ducts immediately bend to the dorsal body surface at the anterior level of the radial muscle plates and, thereafter, show a dorsal loop before entering the penis papilla. This condition is present in the specimens of *C. a. alpina* from Corsica that I examined. However, the animals do not belong to *C. a. corsica* as they possess body pigmentation and/or a comparatively small copulatory complex, as well as smaller testes, which only extend dorsally to, or a little beyond, the median line of the body.

In view of the taxonomic status of the other forms (see below), the nominate form is best considered to represent its own, separate species, *C. alpina*, and not a subspecies.

8.2.2. *Crenobia a. septentrionalis*

This monopharyngeal form in the northern part of the range is merely characterized by asexual reproduction through the process of fission. However, this may merely “reflect the fact that more northerly habitats are in general more favourable to asexuality” (Baird 1983: 15). Therefore, the nomen of this taxonomically poorly defined form should be suppressed and the northern asexual populations should simply be assigned to the species *C. alpina*.

8.2.3. *Crenobia a. corsica*

8.2.3.1. Material examined. RMNH VER. 21014.a, spring above Lac de

Melu, Corsica, France, 42°12.834 N – 9°01.178E, 18 April 2015, alt. 1718 m, snow cover 90–100%, coll. H. Smit, sagittal sections on 5 slides; RMNH VER. 21014.b, *ibid.*, sagittal sections on 4 slides.

It is noteworthy that syntypes of this form are still available at the Museum für Naturkunde in Berlin, under catalogue numbers 6362 (5 specimen in ethanol), and 9729–9736 (59 slides in total).

8.2.3.2. Comparative description and discussion. In contrast to the above-mentioned taxa, this is a purely white form. It is in possession of eyes and a single pharynx and has its mouth opening located at the most posterior end of the pharyngeal pocket. According to Arndt (1922), live specimens measure up to 11 mm in length, while the body of preserved animals is up to 7.5 mm long and 2.5 mm wide, with most of the sexually mature, preserved specimens measuring 5–6 x 1 mm. This agrees with the dimensions of preserved specimen RMNH VER. 21014.b, which measured about 6.5 x 1.6 mm.

Although this is a rather small form, its copulatory apparatus and testicular follicles are comparatively large. The large size of the testes was already mentioned by Arndt (1922) and I found in my material that the prepharyngeal follicles occupy most of the dorso-ventral space (Fig. 11A). In relation to the relatively small size of the body, the copulatory complex is very large, occupying a major part of the body space in anterior-posterior direction, at least in preserved specimens (Fig. 11B). In contrast to all other forms, the entire system of muscle plates, fibrous tissue, and outer coat of myoblasts is not more or less ball-shaped, but is distinctly barrel-shaped (Figs. 13 and 14). Despite the comparatively large size of this barrel-shaped portion of the copulatory complex, the penis papilla is relatively small, as it only reaches just over halfway the length of the anterior portion of the genital atrium (Figs. 12 and 13).

The vasa deferentia recurve at about the level of the atrial fold and also show a distinct dorsal loop before penetrating the muscle layer and then uniting to form the ejaculatory duct. The trajectories of the sperm ducts are symmetrical.

Although Arndt (1922) considered this form to be widely distributed across Corsica, he found the specimens mostly at the still higher reaches of the streams, as compared with the distribution of the also cold-loving nominate form. In that respect, it is noteworthy that our specimens were collected from a spring at high altitude (1718 m), while the snow cover was 90–100%.

The anatomical characters of *corsica* set this form apart from all other forms and support its status as a separate species. This notion is supported by a molecular analysis of *C. alpina* populations from the European Alps that included also specimens from Corsica. In the resulting phylogenetic tree, the specimens from Corsica formed a separate clade

that did not group with *Crenobia* populations from the Alps (Brändle et al. 2017).

8.2.4. *Crenobia a. montenigrina*

8.2.4.1. Material examined. RMNH VER. 21015.a, Prionia, Greece, coll. H. Malicky, sagittal sections on 2 slides; RMNH VER. 21015.b, *ibid.*, sagittal sections on 4 slides; RMNH VER. 21016.a, Andros, Apikia, Greece, coll. H. Malicky, sagittal sections on 4 slides; RMNH VER. 20020.a, east of Ag. Ioannis, Crete, Greece, coll. H. Malicky, sagittal sections on 2 slides; RMNH VER. 20035.a, Roussakiana, Crete, Greece, 35.211976N–25.515804E, alt. 935 m, 6 April 2009, coll. E. Mateos & E. Solà, sagittal sections on 8 slides; RMNH VER. 20036.a, *ibid.*, sagittal sections on 7 slides; RMNH VER. 20036.b, *ibid.*, sagittal sections on 6 slides; ZMA V.Pl. 740.1, Meso Potami, Crete, Greece, 23 September 1972, coll. H. Malicky, sagittal sections on 4 slides; ZMA V.Pl. 740.2, *ibid.*, sagittal sections on 4 slides; ZMA V.Pl. 747.1, Meso Potami, Crete, Greece, 16 December 1981, coll. H. Malicky, sagittal sections on 3 slides; ZMA V.Pl. 747.2, *ibid.*, sagittal sections on 2 slides; ZMA V.Pl. 733.1, Meso Potami, Crete, Greece, 25 February 1982, coll. H. Malicky, sagittal sections on 3 slides; RMNH VER. 20016.a, Meso Potami, Crete, Greece, coll. H. Malicky, sagittal sections on 6 slides; RMNH VER. 20017.a, Meso Potami, Crete, Greece, coll. H. Malicky, sagittal sections on 5 slides; ZMA V.Pl. 733.2, Meso Potami, Crete, Greece, 23 February 1982, coll. H. Malicky, sagittal sections on 5 slides; RMNH VER. 21017.a, Protoria, Crete, Greece, coll. H. Malicky, sagittal sections on 2 slides; RMNH VER. 21018.a, Gambaralle, Abruzzos, Italy, coll. S. D'Aniello, sagittal sections on 10 slides; RMNH VER. 21018.b, *ibid.*, sagittal sections on 7 slides; RMNH VER. 21019.a, Torrente Tordi, Sicily, Italy, coll. H. Malicky, sagittal sections on 2 slides; RMNH VER. 20018.a, Torrente Tordi 2, Sicily, Italy, coll. H. Malicky, sagittal sections on 4 slides; RMNH VER. 20019.a, N. Petralia, Sicily, Italy, sagittal sections on 4 slides; RMNH VER. 21020.a, spring at F. Sagittario, 20 October 1986, sagittal sections on 5 slides; RMNH VER. 21020.b, *ibid.*, sagittal sections on 5 slides; RMNH VER. 21020.c, *ibid.*, sagittal sections on 5 slides.

8.2.4.2. Comparative description and discussion. Generally, its polypharyngeal condition has been considered as the diagnostic and defining character of this form. In addition to the first, central or primary pharynx, there is a large number of additional pharynges, which occupy more lateral positions in the pharyngeal pocket. The total number of pharynges generally varies between 11 and 17, although, according to Chichkoff (1903), Bulgarian specimens may have 23–34 pharynges. Due to the large number of additional pharynges, the pharyngeal pocket is very large and may occupy almost one-third of the

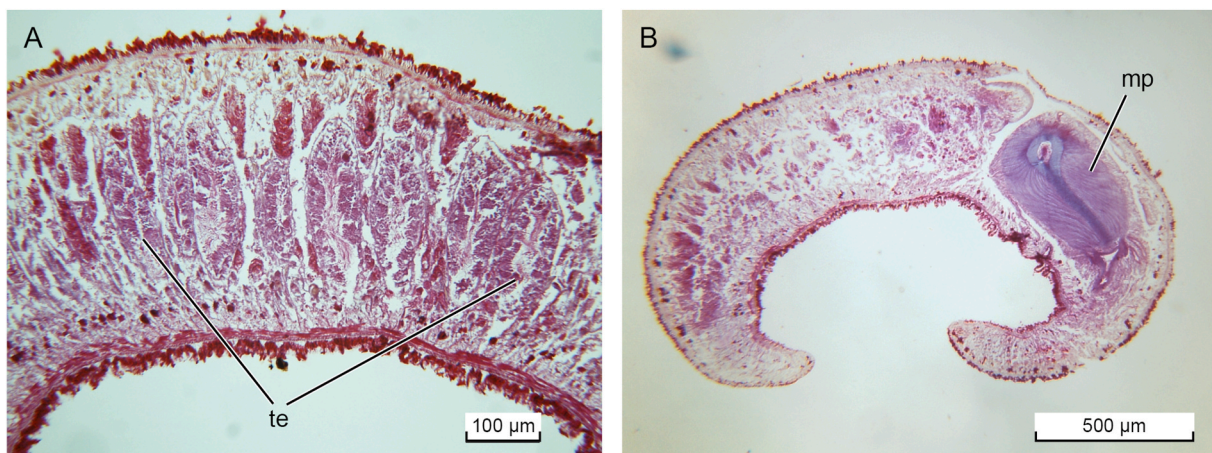


Fig. 11. *Crenobia a. corsica*. Photomicrographs. (A) RMNH VER. 21014.a, sagittal section, showing the large testes (B) RMNH VER. 21014.a, sagittal section, showing the large copulatory complex.

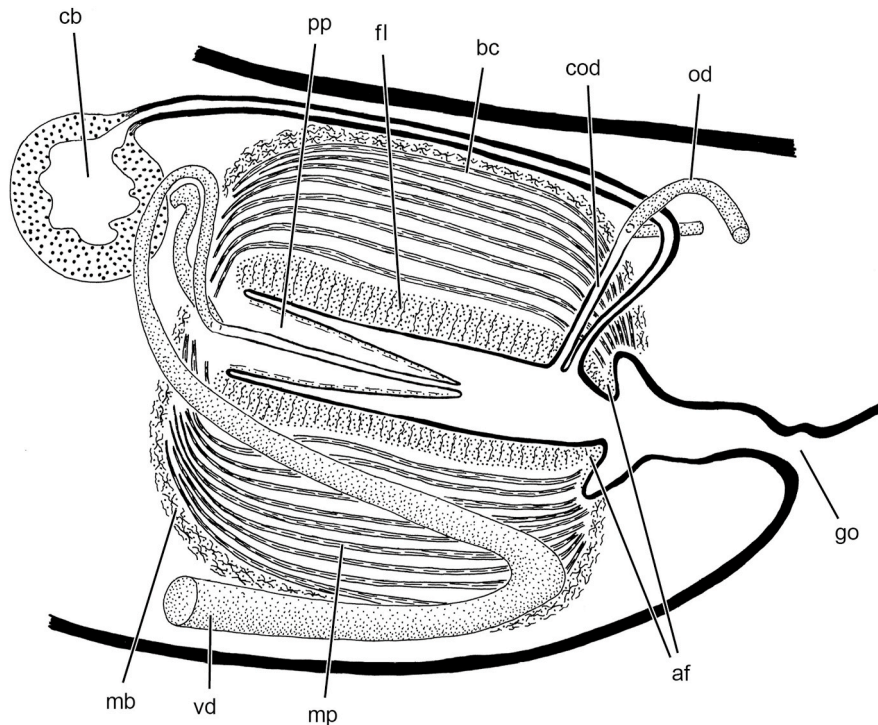


Fig. 12. *Crenobia a. corsica*. Diagrammatic reconstruction of the copulatory apparatus.

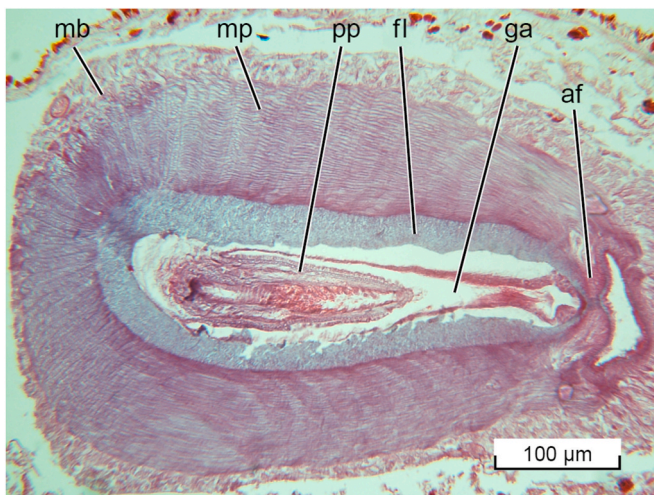


Fig. 13. *Crenobia a. corsica*. RMNH VER. 21014.a, sagittal section. Photomicrograph of penis papilla, in relation to size atrium.

body length (Mrázek 1904; Steinmann 1909). Previous workers apparently failed to notice that in *montenigrina* the mouth opening is not located at the very posterior end of the pharyngeal pocket, as in the other forms, but that it has shifted anteriorly for some distance (Fig. 14).

Other characteristic features of *montenigrina* concern the testes. The ventrally located testicular follicles are rather large (Beauchamp 1932; Gourbault 1972), so that their dorsal portion may extend to, or even beyond, the median line of the body (Fig. 15). Furthermore, the testes extend backwards for some distance posterior to the root of the first, central pharynx, as already correctly observed by Steinmann (1907, 1909), Komárek (1919), Beauchamp (1932), and Stella (1957) (Fig. 15). This contrasts with the strictly prepharyngeal distribution of the testes in the other forms.

In *montenigrina*, the trajectories of the vasa deferentia are

symmetrical and are characterized by a pronounced recurvature at the level of the atrial fold and a distinct dorsal loop before the ducts unite at the base of the penis papilla (Figs. 14 and 16). The pronounced recurvature of the sperm ducts was already clearly described, as well as depicted by Chichkoff (1903: fig. 3), while one of his other drawings suggests the presence of dorsal loops (Chichkoff 1903: fig. 2).

The penis papilla is rather large and usually projects through the atrial fold. In some specimens the copulatory bursa has acquired the shape of an elongated sac (Fig. 16).

On the basis of these anatomical features, I conclude that *montenigrina* is clearly differentiated from the other taxa of *Crenobia* and, thus, represents a full species. This separate specific status is supported by Steinmann's (1909) unsuccessful attempts to cross specimens of nominate *C. alpina* with specimens of *montenigrina*, while he observed in a pure culture of *montenigrina* many copulating individuals. Furthermore, in a molecular phylogenetic analysis, the operational taxonomic unit concerning *montenigrina* occupied its own, separate branch, albeit that it grouped among clades from the European Alps (Brändle et al. 2017).

8.2.5. *Crenobia a. anophthalma*

8.2.5.1. *Material examined.* RMNH VER. 21021.a, Cigina Jama, Dinara Mt., Croatia, 43°48.538N-16°45.763E, alt. 1130 m, 28 March 2015, coll. Tonci Radja, sagittal sections on 4 slides; RMNH VER. 21021.b, *ibid.*, sagittal sections on 3 slides; RMNH VER. 21021.c, *ibid.*, sagittal sections on 5 slides; RMNH VER. 21022.a, Vuena Jama, Vrdovo Ravno, Croatia, 43°50.345N-16°38.897E, alt. 940 m, 24 May 2015, coll. Tonci Radja, sagittal sections on 3 slides; RMNH VER. 21022.b, *ibid.*, sagittal sections on 3 slides; RMNH VER. 21022.c, *ibid.*, sagittal sections on 2 slides; RMNH VER. 21022.d, *ibid.*, sagittal sections on 3 slides; RMNH VER. 21022.e, *ibid.*, sagittal sections on 4 slides; RMNH VER. 21022.f, *ibid.*, sagittal sections on 3 slides; RMNH VER. 21022.g, *ibid.*, sagittal sections on 3 slides.

8.2.5.2. *Comparative description and discussion.* According to Mrázek (1907), *anophthalma* is similar to *alpina* in its external form and size.

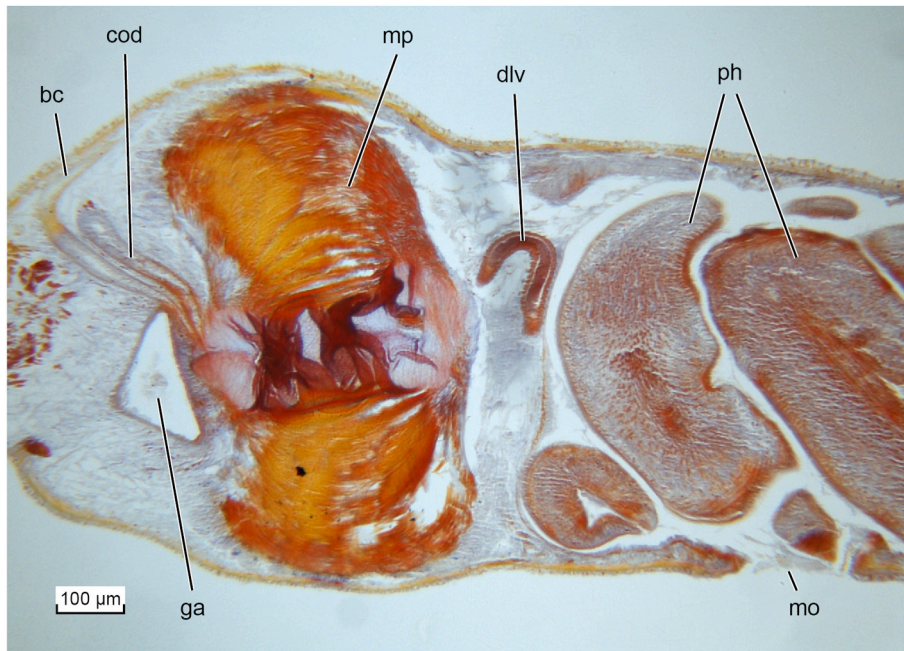


Fig. 14. *Crenobia a. montenigrina*. RMNH VER. 20017.a, sagittal section. Photomicrograph of mouth opening shifted anteriorly, and copulatory complex with dorsal loop of vas deferens.

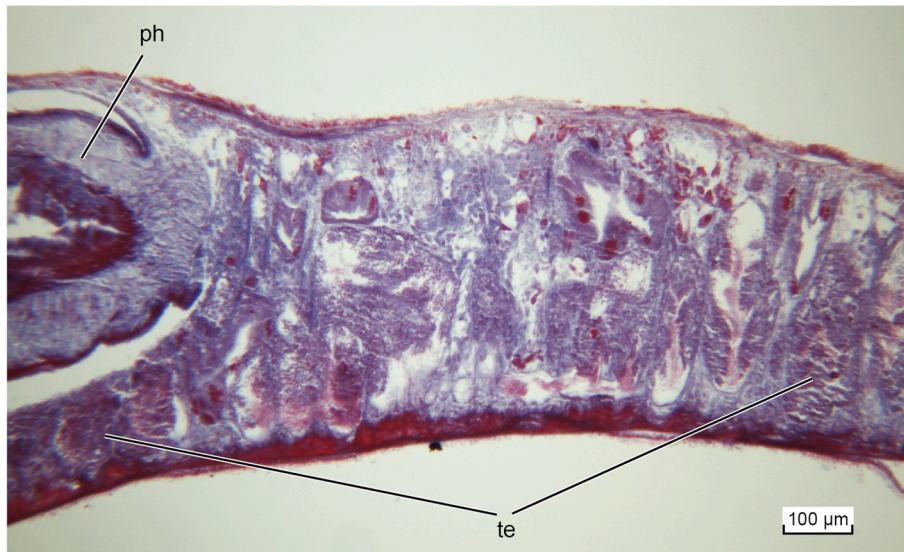


Fig. 15. *Crenobia a. montenigrina*. RMNH VER. 20036.b, sagittal section. Photomicrograph of large testes, extending backwards beyond the root of the pharynx.

Preserved specimens available to me measured up to 4.5 mm in length and 1.5 mm in width. The animals are completely white, unpigmented, and devoid of eyes.

Although this form is polypharyngeal, it never has more than three pharynges in total. In addition to the central, primary pharynx there are two extra, slightly smaller pharynges (Fig. 17A). Most specimens, even rather small ones, have three pharynges; occasionally, specimens may show only two pharynges. Due to the smaller number of pharynges, the pharyngeal pocket is much smaller than in polypharyngeal *montenigrina*. The mouth opening is located at the most posterior end of the pharyngeal pocket. The ventrally located, prepharyngeal testes are rather large (Fig. 17B). The long and slender penis papilla generally extends beyond the atrial fold.

Previous workers failed to notice that also in *anophthalma* the trajectories of the vasa deferentia are highly asymmetrical. One of the

sperm ducts runs backwards to about halfway the level of the anterior portion of the genital atrium or may even extend to a level well beyond the atrial fold (Fig. 18). From thereon, this sperm duct exhibits a highly arched course in antero-dorsal direction. Then, at about the level of the root of the penial papilla, the duct curves downwards and, thereafter, bends again in posterior direction. Immediately hereafter, it penetrates the muscular coat around the atrium and unites with the other sperm duct to form the ejaculatory duct (Fig. 18).

The course of the other sperm duct is quite different, in that it immediately turns dorsad after having reached the anterior level of the muscular coat around the atrium. Further, it exhibits a dorsal loop before it curves towards the base of the penial papilla and unites with the other vas deferens to form the ejaculatory duct (Fig. 18).

That the presence of maximally three pharynges can be considered to represent a stable, species-specific character is supported by the

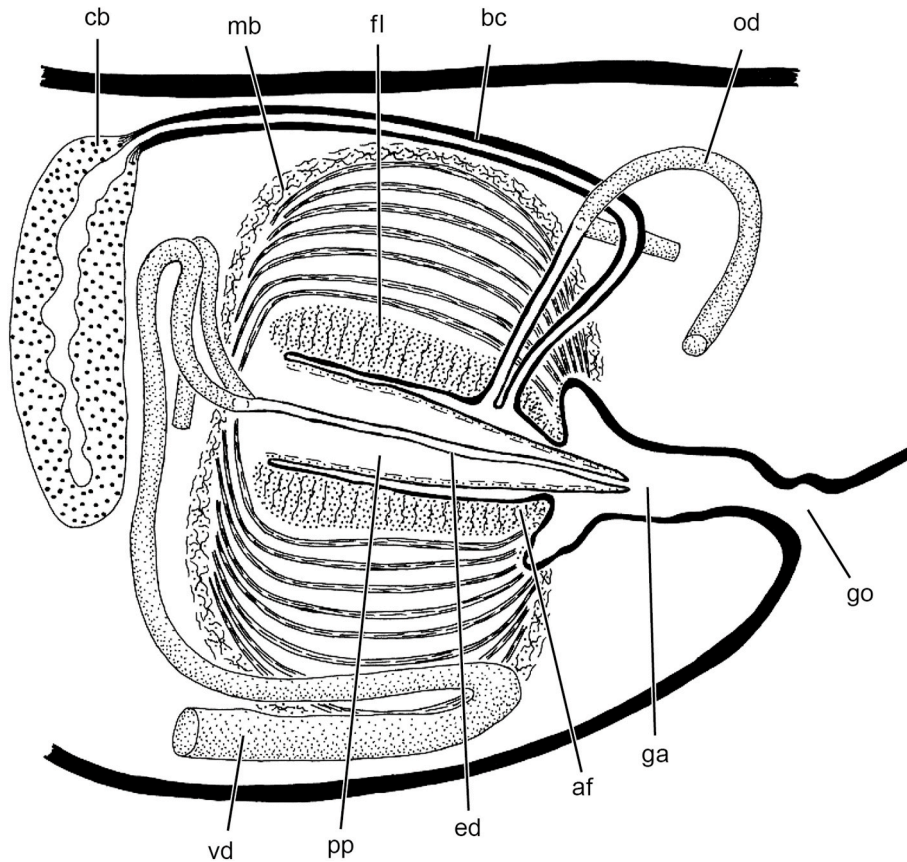


Fig. 16. *Crenobia a. montenigrina*. Diagrammatic reconstruction of the copulatory apparatus.

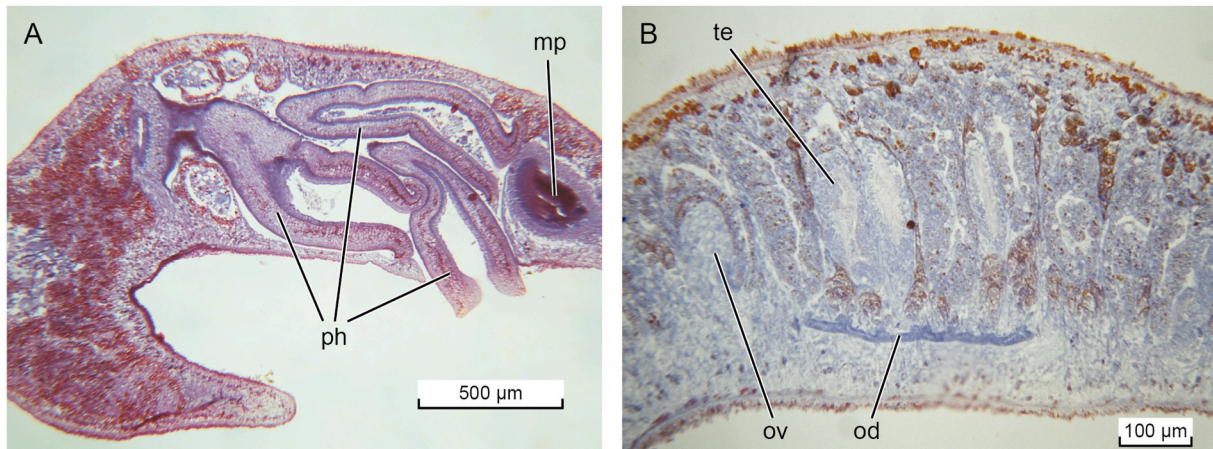


Fig. 17. *Crenobia a. anophthalma*. Photomicrographs. (A) RMNH VER. 21021.a, sagittal section, showing the three pharynges (B) RMNH VER. 21022.b, sagittal section, showing the rather large testes.

regeneration experiments of Mrázek (1914). He found that in *anophthalma* the animal always regenerated three pharynges, while in experiments with nominate *alpina* none of the experimental animals abnormally regenerated more than one pharynx. In combination with the lack of eyes, unpigmented body, and the asymmetrical trajectories of the sperm ducts, this forms ample evidence for the separate species status of *anophthalma*.

8.2.6. *Crenobia a. bathycola*

This is a small, unpigmented form that, in contrast to *anophthalma*, has only one pharynx and possesses two eyes. Body size of sexually

mature specimens ranges between 3.2 and 5.4 mm in length and 0.8–1.5 mm in width; body length of the majority of the specimens examined ranged between 3.5 and 4.5 mm (Steinmann 1911; Schmassmann 1923).

The eyecups are shallow and frequently give the impression of being merely a weakly curved plate, in contrast to the hemispherical pigment cups in nominate *alpina* (Schmassmann 1923).

The 20–24 prepharyngeal testes are well-developed and rather large, measuring 180–210 µm in diameter, occasionally even 250 µm, and occupy the entire dorso-ventral space (Steinmann 1911; Schmassmann 1923).

In comparison with its small body size, the copulatory complex is

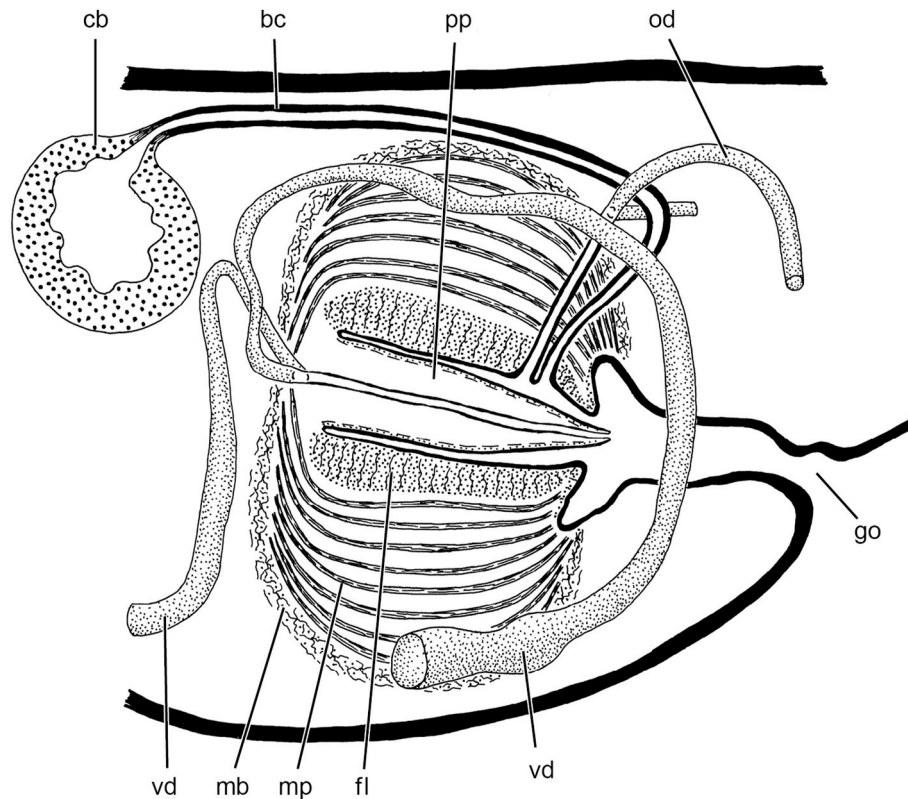


Fig. 18. *Crenobia a. anophthalma*. Diagrammatic reconstruction of the copulatory apparatus.

very large, with the dimensions of the muscular coat around the atrium being $244 \times 166 \mu\text{m}$, while occupying the entire dorso-ventral space; the penis papilla is a plump, cone-shaped structure, measuring $39 \mu\text{m}$ in diameter (Schmassmann 1923).

The ecology of *bathycola* is completely different from that of any of the other taxa of *Crenobia*, in that it lives in the muddy bottoms of lakes, at depths varying between 7 and 280 m (Steinmann 1911; Schmassmann 1923; Steinböck 1949). With respect to its anatomy, *bathycola* resembles *corsica* in the presence of large, dorso-ventral testes, eyes, a very large copulatory complex, and in the absence of pigmentation. However, in *corsica* the eyecups are not shallow, but show their usual shape, which lies somewhere between ball-shaped and hemispherical. Taking everything into consideration, I conclude that *bathycola* also deserves full species status.

9. Conclusions

The genus *Crenobia* ranges across Northern, Western, Southern, and Eastern Europe, including several islands in the Mediterranean region, but it is absent from a major part of the Iberian Peninsula; it is distributed also in Turkey. In the past, different views have been expressed by various workers on the taxonomic status of several geographic and anatomical forms, which were ranked as either forms, varieties, subspecies, or as full species. It is here shown that the genus *Crenobia* comprises five, well-defined full species, viz., *C. alpina*, *C. corsica*, *C. montenigrina*, *C. anophthalma*, and *C. bathycola*. The former subspecies *C. alpina septentrionalis* should be suppressed, as it merely represented asexually reproducing northern populations of *C. alpina*.

Acknowledgements

I am grateful to Harry Smit for making available specimens from Corsica, to Tonci Radja for material from Croatia, to Eduardo Mateos

and Eduard Solà for providing animals collected on Crete, and to Miquel Vila-Farré for providing specimens from Spain and Italy. Birger Neuhaus kindly provided information on the registration numbers of the syntypes of *Crenobia corsica* deposited in the Museum für Naturkunde in Berlin. I am grateful to Menno Hooft (Naturalis Biodiversity Center, Leiden) for scanning the drawings for Figs. 10, 12, 16 and 18 and to Henk Caspers (Naturalis Biodiversity Center, Leiden) for the digital rendering of all figures.

References

- Adlmanseder, A., 1953. Die geographische Verbreitung der *Planaria alpina* und einiger anderer Eiszeitrelikte im oberösterreichischen Alpenvorland unter besonderer Berücksichtigung des Flußgebietes der Antiesen. 81. Jahresbericht des Bundesgymnasiums u. -Real-Gymnasiums Ried i. Innkr./O.-O. am Schlusse des Schuljahres 1952/53, 12–20.
- Arndt, W., 1922. Untersuchungen an Bachtricliden. Ein Beitrag zur Kenntnis der Paludicolen Korsikas, Rumäniens und Sibiriens. Z. wiss. Zool. 120, 98–146. + 1 pl.
- Arndt, W., 1926. 2. Turbellaria. Mitt. Zool. Mus. Berlin 12, 218–222.
- Arrow, 2019. Český Hydrometeorologický Ústav. http://hydro.chmi.cz/isarrow/objects.php?objekty_biodata=1&taxon=Crenobia%20alpina. (Accessed 28 March 2019).
- Babalean, A.F., 2017. A New Record of *Crenobia alpina* (Dana, 1776) (Platyhelminthes, Tricladida) in Romania. Philica.com Article number 1107.
- Baird, D.J., 1983. The Population Biology of *Crenobia alpina* (Dana). Unpublished PhD Thesis. University of Glasgow, p. 158.
- Ball, I.R., Reynoldson, T.B., 1981. British Planarians. Cambr. Univ. Press, Cambridge. VI + 141 pp.
- Beauchamp, P. de, 1932. Biospeologica LVIII. Turbellariés, Hirudinees, Branchiobdellidés. Arch. Zool. Exp. Gen. 73, 113–380 + 2 pls.
- Beauchamp, P. de, 1961. Classes des Turbellariés. In: Grassé, P.P. (Ed.), Traité de Zoologie IV. Masson et Cie, Paris, pp. 35–212.
- Benazzi, M., 1946. *Planaria alpina* nell'isola d'Elba. Atti Acad. Fisiocritici, Sez. Agraria 11, 1–7.
- Benazzi, M., 1947. Presenza della *Planaria alpina* nelle Alpi Apuane. Att. Soc. Toscana Sc. Nat. 54, 68–69.
- Benazzi, M., 1961. Les planaires des îles Tyrrhéniennes et leur différenciation raciale, cytologique et génétique. In: Le peuplement des îles Méditerranéennes et le problème de l'insularité. CNRS, Paris, pp. 103–112.
- Benazzi, M., Benazzi Lentati, G., 1976. Animal Cytogenetics 1 Platyhelminthes. Gebr. Borntraeger, Berlin, Stuttgart, p. 182 + V.

- Beracko, P., Revajová, A., 2019. Benthic life in karst spring – the life cycle and secondary production of benthic macroinvertebrates under the effects of constant water temperature. *Limnologia* 74, 51–60.
- Brändle, M., Heiser, R., Marten, A., Brandl, R., 2007. Population structure of the freshwater flatworm *Crenobia alpina* (Dana): old lineages and low gene flow. *J. Biogeogr.* 34, 1183–1192.
- Brändle, M., Sauer, J., Opgenoorth, L., Brandl, R., 2017. Genetic diversity in the Alpine flatworm *Crenobia alpina*. *Web Ecol.* 17, 29–35.
- Chichkoff, G., 1903. Sur une nouvelle espèce du genre *Phagocata* Leidy. *Arch. Zool. Expér. et Gén.*, sér. 4 (1), 401–405. + 1 pl.
- Codreanu, R., 1956. Raspindirea unei planarii polifaringiene (*Crenobia alpina montenigrina*/Mrázek/1903 in Carpații Sudici. *Anal. Inst. de Cerc. Pisc.* 1, 385–399.
- Dahm, A.G., 1958. Taxonomy and Ecology of Five Species Groups in the Family Planariidae (Turbellaria Tricladida Paludicola). *Nya Litografen, Malmö*, p. 241.
- Dahm, A.G., Gourbault, N., 1978. Tricladida et Temnocephalida (Turbellaria). In: Illies, J. (Ed.), *Limnofauna Europaea*, Gustav Fischer Verlag. Gustav Fischer Verlag, Stuttgart & New York, Zwets & Zeitlinger B.V., Amsterdam, pp. 16–20.
- Dana, J.P.M., 1766. De Hirudinis nova specie, noxa, remediisque adhibendis. *Mél. de Phil. et de Math. de la Soc. Roy. de Turin* 3, 199–205 + Plate 6.
- de Queiroz, K., 1998. The general lineage concept of species, species criteria, and the process of speciation. In: Howard, D.J., Berlocher, S.H. (Eds.), *Endless Forms – Species and Speciation*. Oxford Univ. Press, Oxford, pp. 57–75.
- de Queiroz, K., 2007. Species concepts and species delimitation. *Syst. Biol.* 56, 879–886.
- Durance, I., Ormerod, S.J., 2010. Evidence for the role of climate in the local extinction of a cool-water triclad. *J. North Am. Benthol.* Soc. 29, 1367–1378.
- Dutrillaux, B., Lénicque, P., 1971. Analyse du caryotype de cinq espèces de planaires par la méthode du choc hypotonique. *Acta Zool* 52, 241–248.
- Fülep, T., 2009. Study on the occurrence of Turbellaria species (Platyhelminthes: Turbellaria) in the small watercourse-system of Baróc-Patak in the valleys (Torma-Völgy, Moldva-Völgy, Recem-Völgy, Vár-Völgy, Baróc-Völgy) of the mountains Bükk, northeastern Hungary. *Acta Biol. Debr., Oecol. Hung.* 20, 99–106 (in Hungarian).
- Geissen, H.-P., 1993. *Crenobia alpina* (DANA) (Turbellaria: Tricladida) weiter am Hunsrückrand. *Fauna u. Flora in Rheinland-Pfalz* 7, 185–187.
- Giesen-Hildebrand, D., 1975. Die Planarienfauna der Siebengebirgsbäche – eine ökologisch-historische Untersuchung unter besonderer Berücksichtigung der Befunde von Voigt (1894). *Decheniana* 128, 21–29.
- Gourbault, N., 1972. Recherches sur les triclades paludicoles hypogés. *Mém. Mus. Nat. Hist. Nat. sér. A* 73, 1–249 + 3 pls.
- Hauser, J., 1953. Zur Rheophilie von *Planaria alpina*. *Zool. Anz.* 151, 73–74.
- Hauser, J., Heller, Z., 1983. Augenmorphologie von *Crenobia alpina* (Dana, 1976) – (Turbellaria, Tricladida). *Acta Biol. Leopoldensia* 5, 209–220.
- Hoffmann, J.A., 1964. Faune des triclades paludicoles du Grand-Duché de Luxembourg. *Institut Grand-Ducal de Luxembourg, Sect. des Sci. nat. phys. et mathém., Archives, nouv. sér.* 30, 181–261.
- Hofsten, N. von, 1916. Turbellarien der nordschwedischen Hochgebirge. *Naturwiss. Unters. D. Sarekgebirges in Schwedisch-Lapland, IV Zoologie*, pp. 697–742.
- Hofsten, N. von, 1919. *Planaria alpina* som glacialrelik på Bornholm jämte några ord om landfaunans invandring till Bornholm. *Vidensk. Medd. Fra Dansk naturh. Foren.* 71, 39–75.
- Horstmann, D., 2013. Zum Vorkommen und zur Verbreitung der Planarien im südlichen Teutoburger Wald und Eggegebirge unter besonderer Berücksichtigung der Alpenplanarie (*Crenobia alpina*). *Abh. Westfälischen Mus. Naturk.* 75, 161–172.
- Ischreyt, G., 1927. Ueber das Vorkommen der *Planaria alpina* Dana in Kurland. *Arch. f. Hydrobiologie* 18, 129–132.
- Kennel, J., 1888. Untersuchungen an neuen Turbellarien. *Zool. Jahrb., Abh. Anat. Ontog.* Tiere Bd. 3, 447–486 + 2 pls.
- Kenk, R., 1930. *Euplanaria cretica* Meixner, eine Triklade mit eigentümlichem Drüsenorgan. *Zool. Anz.* 92, 247–253.
- Kenk, R., 1974. Index of the genera and species of the freshwater triclad (Turbellaria) of the world. *Smithsonian Contrib. Zool.* 183, 1–90.
- Kenk, R., 1978. The planarians (Turbellaria: Tricladida: Paludicola) of Lake Ohrid in Macedonia. *Smithsonian Contrib. Zool.* 280, 1–56.
- Komárek, J., 1919. O temnostních Tricladách (Vermes, Turbellaria) z krásy balkánských na základě sběru Dra. Karla Absolona I. *Casopis Moravského Musea zemského* 16, pp. 255–304 + 3 pls.
- Komárek, J., 1920. Über höhlenbewohnende Tricladen der balkanischen Karste (Gesammelt von Dr. K. Absolon.). *Arch. f. Hydrobiol.* 12, 822–828.
- Kosswig, C., Ermin, R., 1950. *Crenobia* (Planaria) alpina Dana in Anatolien. *Rev. Fac. Sci. Univ. Istanbul, ser. B* 15 (3), 225–231.
- Lorch, S., Zeuss, D., Brandl, R., Brändle, M., 2016. Chromosome numbers in three species groups of freshwater flatworms increase with increasing latitude. *Ecol. Evol.* 6 (5), 1420–1429.
- Luther, A., 1961. Die Turbellarien Ostfennoskandiens II. Tricladida, vol. 11. *Fauna Fennica*, pp. 1–42.
- Mayr, E., 1969. *Principles of Systematic Zoology*. McGraw-Hill, New York, p. 428 + XI.
- Micoletzky, H., 1907. Eine zweite polypharyngeale planarienform aus Montenegro. *Sitzber. Königl. Böhm. Ges. d. Wiss., Prag* 1906, pp. 18 + 1 pl.
- Mrázek, A., 1904. Über eine neue polypharyngeale Planarienart aus Montenegro (Pl. montenigrina n. sp.). *Věstník královské České Společnosti Náuk. Trida math.-přírod.* 33 (for 1903), p. 43 + 2 pls.
- Mrázek, A., 1907. Eine zweite polypharyngeale planarienform aus Montenegro. *Sitzber. Königl. Böhm. Ges. d. Wiss., Prag* 1906, pp. 18 + 1 pl.
- Mrázek, A., 1914. Regenerationsversuche an der tripharyngealen *Planaria anophthalma*. *Arch. Entwickl.mech. der Organismen* 37, 252–276.
- Năstăsescu, M., 1974. Triclade din România, studiu morfologic, systematic, ecologic și zoogeographic – Rezumatul tezei de doctorat. *Universitatea din București, Facultatea de Biologie, București*, p. 51.
- Okoń, D., Bionńska, A., Różkowski, J., Wojtal, A.Z., 2020. Natural and anthropogenic factors controlling the calcareous springs biodiversity (Cracow-Częstochowa Upland, Poland). *Ecohydrol. Hydrobiol.* 20, 644–659.
- Oomen, H.C.J., Geelen, J.F.M., 1966. On the distribution of *Crenobia* (Planaria) alpina (Dana) in The Netherlands. *Arch. Néerl. Zool.* 16, 440–452.
- Osborne, R., Tarling, D., 1995. *The Viking Historical Atlas of the Earth*. Viking, Middlesex, p. 192.
- Pala, M., Casu, S., Vacca, R.A., 1980. Sulla presenza di “*Crenobia alpina* (Dana)” (Turbellaria, Tricladida) in Sardegna. *Boll. Soc. Sarda Sci. Nat.* 19, 171–175.
- Pagast, F., Froese, H., 1933. Beitrag zur Kenntnis der Quellenfauna Lettlands. *Inst. f. wiss. Heimatforschung an der Livländ. Gemeinnützigen u. Ökon. Soz. in Dorpat, Mitt.* 9, 1–32.
- Petersen, M., 1935. *Planaria alpina* in Ostpommern. *Dohrniana* 14, 92–100.
- Poliński, W., 1926. Observations écologiques sur *Planaria alpina* et *Pl. gonocephala* en Pologne. *Ann. Zool. Musei Polonici* 5, 22–51.
- Rhön, 2022. *Alpine Planaria*. <https://rhoen.quellen-grundwasser.de/en/alpenstrudelwurm.html>. (Accessed 11 March 2022).
- Roca, J.R., Ribas, M., Bagaña, J., 1992. Distribution, ecology, mode of reproduction and karyology of freshwater planarians (Platyhelminthes: Turbellaria; Tricladida) in the springs of the central Pyrenees. *Ecography* 15, 373–384.
- Roszkowski, W., 1921. Kilka nowych stanowisk wyplawków krynicznych. *Kosmos, Bull. Soc. polonaise Naturalistes* 46, 639–649.
- Roszkowski, W., 1930. Third note on *Planaria alpina* and *Planaria gonocephala* in the vicinity of Ojców. *Fragm. Daubustica Musei Zool. Polonici* 1, 146–151.
- Schmassmann, W., 1923. Die Bodenfauna hochalpiner Seen. *Arch. Hydrobiol. (Plankt.)*, Suppl. Bd. 3, 1–106 + plate 1.
- Sluys, R., 1991. Species concepts, process analysis, and the hierarchy of nature. *Experientia* 47, 1162–1170.
- Sluys, R., Hazevoet, C.J., 1999. Pluralism in species concepts: dividing nature at its diverse joints. *Species Divers.* 4, 243–256.
- Soós, Á., 1943. Beiträge zur Kenntnis der Verbreitung von *Crenobia alpina*, *Euplanaria gonocephala* und *Polycelis cornuta* in Ungarn. *Ann. Hist.-nat. Mus. Hung. Pars Zool.* 36, 25–33.
- Steinböck, O., 1942. Das Verhalten von *Planaria alpina* Dana in der Natur und im Laboratoriumversuch. *Mem. Ist. Ital. Idrobiol. Dott. Marco Marchi* I, 63–75.
- Steinböck, O., 1949. Zur Turbellarien-Fauna des Lago Maggiore und des Lago di Como. *Mem. Ist. de Marchi* 5, 231–254.
- Steinmann, P., 1907. Eine polypharyngeale Planarie aus der Umgebung von Neapel. *Zool. Anz.* 32, 364–366.
- Steinmann, P., 1908. Untersuchungen über das Verhalten des Verdauungssystem bei der Regeneration der Tricladen. *Arch. Entwickl.mech. d. Organismen* 25, 523–568.
- Steinmann, P., 1909. Untersuchungen an neuen Turbellarien. *Z. wiss. Zool.* 93, 157–184 + 1 pl.
- Steinmann, P., 1911. Revision der Schweizerischen Tricladen. *Rev. Suisse Zool.* 19, 175–234.
- Steinmann, P., Bresslau, E., 1913. *Die Strudelwürmer (Turbellaria)*. Verlag von Dr. Werner Klinkhardt, Leipzig, p. 380 + XI.
- Stella, E., 1957. Prime osservazioni su una planaria polifaringea rinvenuta nella sorgente Peschiera. *Boll. Zool.* 24, 227–233.
- Stocchino, G.A., 2018. 80 years of research on planarians (Platyhelminthes, Tricladida) from Sardinia, Italy: an annotated checklist. *Zootaxa* 4532, 539–552.
- Thienemann, A.G., 1938. Rassenbildung bei *Planaria alpina*. In: *Jubiläumsschrift bei Grigore Antipa*. Bukarest, pp. 641–661.
- Thienemann, A., 1950. Verbreitungsgeschichte der Süßwassertierwelt Europas. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, p. 809 + XVI.
- Ulyott, P., 1936. A note on the zoogeographical history of North Western Europe. *Proc. Prehist. Soc., New Series* II, 169–177 + pl. XXXVI.
- van der Velde, G., Cuppen, H.P.J.J., 1981. The distribution and ecology of ground water and rheophilous freshwater triclad (Platyhelminthes, Turbellaria) in The Netherlands. *Nieuwsbrief Europ. Invert. Survey – Nederland* 10, 89–98.
- Vandel, A., 1922. Recherches expérimentales sur les modes de reproduction des planaires triclades paludicoles. *Bull. Biol. France Belg.* 55, 343–518.
- van der Linden, A.G.J.M., 1969. Chromosome number and ploidy-level in a Dutch population of *Crenobia alpina* Dana (*Planaria*). *Genetica* 40, 61–64.
- van Oye, E.L., 1935. *Planaria alpina* Dana var. *alba* nov. var. *Zool. Anz.* 112, 264–268.
- Vejdovsky, F., 1890. Note sur une nouvelle planaire terrestre (*Microplana humicola* nov. gen. nov. sp.) suivi d'une liste des dendrocoeles observés jusqu'à présent en Bohême. *Revue Biol. Nord de la France* 2, pp. 129–148 + pls II, III.
- Vila-Farré, M., Rink, J.C., 2018. In: Rink, J.C. (Ed.), *Planarian Regeneration: Methods and Protocols*, *Methods in Mol. Biol.* 1774. Humana Press, Springer Science+ Business Media, New York, pp. 173–205.
- Volz, W., 1901. Contribution à l'étude de la faune turbellarienne de la Suisse. *Rev. Suisse Zool.* 9, 137–188 + 4 pls.
- Vöfte, A.D., 1929. De Nederlandsche beek-tricladen en de oorzaken van haar verspreiding. *Zuid-Holl. Boek- en Handelsdrukkerij, 's Gravenhage*, p. 116.
- Wegelin, R., 1966. Beitrag zur Kenntnis der Grundwasserfauna des Saale-Elbe-Einzugsgebietes. *Zool. Jahrb., Abt. Syst., Ökol. u. Geogr. Tiere* 93, 1–117.
- Witzleb, W., 1989. Zur Verbreitung der Planarien (Tricladida) im Rhein-Lahn-Kreis, vol. 5. *Fauna Flora Rheinland-Pfalz*, pp. 689–705.
- Zunino, M., Zullini, A., 1995. Biogeografia: la dimensione spaziale dell'evoluzione. *Casa Editrice Ambrosiana, Milano*, p. 310.