Species Distinction and Speciation in Hydrobioid Gastropods (Mollusca: Caenogastropoda: Truncatelloidea)

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Abstract
Hydrobioids, known earlier as the family Hydrobiidae, represent a set of truncatelloidean families whose members are minute, worldwide distributed snails inhabiting mostly springs and interstitial waters. More than 1,000 nominal species bear simple plesiomorphic shells, whose variability is high and overlapping between the taxa, and the soft part morphology and anatomy of the group is simplified because of miniaturization, and unified, as a result of necessary adaptations to the life in freshwater habitats (osmoregulation, internal fertilization and eggs rich in yolk and within the capsules). The adaptations arose parallel, thus represent homoplasies. All the above facts make it necessary to use molecular markers in species discrimination, although this should be done carefully, considering genetic distances calibrated at low taxonomic level. There is common belief in crucial place of isolation as a factor shaping speciation in long-lasting completely isolated habitats. However, neither geomorphological facts (springs are rather ephemeral habitats, there is a net of interstitial habitats which may be used as the ways of expansion), nor the actual genetic structure of (Meta) populations of those stygobionts. Much more than 1,000 nominal species have been described (Figure 1). However, the real number of species is not known, in fact. Not only because of many species to be discovered in the future, but mostly since there are no reliable criteria, how to distinguish a species within the group.

Introduction
Hydrobioids, representing a set of gastropod truncatelloidean families formerly classified as Hydrobiidae, include minute worldwide distributed gastropods whose shells are 1-4 mm high. Some of them inhabit brackish water habitats, some other rivers and lakes, but vast majority are stygobiont, inhabiting springs, caves and interstitial habitats. Nearly nothing is known about the biology and ecology of those stygobionts. Much more than 1,000 nominal species have been described (Figure 1). However, the real number of species is not known, in fact. Not only because of many species to be discovered in the future, but mostly since there are no reliable criteria, how to distinguish a species within the group.

Morphological Characters in Species Distinction
In the Gastropod the shell is the most striking and characteristic structure. The shell remains after the death of animal, thus could be easily studied in old museum materials, or even fossil ones. However, wide ranges of variability of the shells lacking any characteristic feature drastically decrease the usefulness of the shell in species distinction [1-4]. Already, in his classical study on the profundal lymnaeids of the Lake Leman, demonstrated that the shell plasticity definitely excludes, in certain cases, species determination based on the shell: curiously looking shells were found in the gastropods, whose anatomy - especially of reproductive organs - was typical of the two species inhabiting the littoral of the lake [5]. Later, anatomy of the reproductive organs became a basis for all the lymnaeid taxonomy [6]. In hydrobioids, the classical papers of Muus on the Baltic Hydrobiinae demonstrated the usefulness of soft parts: morphology of the penis coupled with head pigmentation, in discrimination of species whose shell variability overlapped [7,8].
Anatomical characters, especially of the reproductive system, became a standard in species-level taxonomy in hydrobioids. The anatomy of the female reproductive system as well as the morphology of the penis was considered in species descriptions and diagnoses [9-23]. On the other hand, there are opinions that between the closely related representatives of the same genus, differences in anatomy are not necessarily present, and, in many cases, would not be expected [24]. The ‘lock-and-key’ mechanism, known mostly in arthropods, is hardly expected in molluscs, whose copulatory organs lack any sclerotized structures, and are more variable than it is usually noted [1,25-28].

In hydrobioids the anatomy does not offer too many characters useful for taxonomy. It is simplified as a result of miniaturisation and made similar because of inevitable adaptations to osmoregulation, internal fertilization, and embryonic development inside a capsule, all necessary in a freshwater habitat, but not plesiomorphic for the Gastropoda. Thus, anatomical similarities are the result of parallelism, or even convergence, at least in many cases, and need not necessarily reflect the common ancestry. In general, homoplasies are common in the Mollusca [29-31].

Species Concepts Applied in Hydrobioid; Introduction of Molecular Data

From among the concepts of species, I prefer the cohesive one proposed by Templeton, not the most often accepted biological one [32,33]. The latter defines species as members of populations that actually or potentially interbreed in nature. This works perfectly in clear situations, where nobody doubts in species distinctness, but is hardly applicable in more complex cases and/or if the data are incomplete. Recent studies on DNA clearly has shown that hybridisation is not as rare as had been previously thought. On the other hand, it is hard to prove hybridisation in nature, and in captivity it may be either blocked or promoted by the conditions unusual for the tested animals. This species concept is also absolutely useless in the case of taxa reproducing asexually (e.g., through parthenogenesis), which are still not less distinct and homogenous than the “normal” species. According to the cohesion species concept, on the other hand, a species is a population, or set of populations, that has genetic or demographic (including ecological niche) cohesion of its members.

Unfortunately, we often know too little about the ecology and biology of the hydrobioid taxa to apply this concept. Often a few specimens or only empty shells - sometimes even broken, uncomplete - can be found, thus the taxonomy is based mostly on the shell morphology. Another widely acknowledged idea is that the gene flow among sympatric species results in their differentiation [27,28]. There are also concepts of strictly geographical, but large-scale patterns of endemism (just a distinct species in each spring/cave, or at least, in each spring/cave complex:) resulted in enormously long list of presumed species [23,25-28,54]. There are also concepts of strictly geographical, but large-scale pattern of vicariant distribution [60].

For example, Bichain et al., reported that the species threshold value of K2P distance in the west European Bythinella was 0.015 [36]. Several numerical techniques for species distinction applying the genetic distances were introduced [46,47]. Applying mitochondrial DNA one should be careful since there are cases of introgression, although not often [48].

Morphostatic Mode of Evolution

In hydrobioids it is common that molecular differences are not well reflected in morphology, which confirms the morphostatic model of evolution, with numerous cryptic species within the genus [40,49]. Morphostatic evolution, as defined by Davis, is often a result of non-adaptive radiation marked by the rapid proliferation of species without ecological differentiation [50,51]. This results in a flock of species that are not differentiated morphologically (and ecologically). Although traditionally neglected, non-adaptive radiation seems not to be a rare phenomenon in gastropods [52,53]. Thus the criterion of morphological differences between molecularly distinct taxa may not be useful in the case of morph static evolution. In practice, it is not easy to assess the distinctness of species in more or less different yet allopatric taxa. Where there is sympathy combined with well-marked differences (morphology included) species distinctness is well supported. At this point, however, one should be more than careful in case of morphology – in springs reproduction takes place throughout a year, but the conditions - like amount of food (e.g., algae) varies between summer and winter, which often results in generations strikingly different in morphology at the same spring, which mimics distinct species [54].

Ways of Speciation

The crucial point in distinguishing species is our knowledge - if any - about the ways of speciation in the considered group. And at this point, in hydrobioids we have much more myths and legends than real knowledge. Wide ranges of variability of the shells lacking any characteristic feature, coupled with common belief in extremely high levels of endemism (just a distinct species in each spring/cave, or at least, spring/cave complex:) resulted in enormously long list of presumed species for the Bulgarian stygobiont gastropods [23,55,56]. There are popular speculations about the founder effect in Hydrobioids, but they are not supported by the genetic structure of local populations, not resembling the one expected as a consequence of founder effect [57-59]. There are also concepts of strictly geographical, but large-scale pattern of vicariant distribution [60].

Undoubtedly the levels of endemism are overestimated, and type specimens stored as empty shells are hardly helpful in solving problems with identification and species boundaries in the hydrobioid snails. In stygobiont hydrobioids living snails can be collected as single specimens, only exceptionally a few specimens can be collected at a place, thus the molecular data, useful in phylogeny as well as in the studies on genetic structure of population, only recently begun to accumulate [61]. It is commonly believed that the springs inhabited by those obligatorily aquatic animals are stable and long lasting habitats. Certainly this cannot be truth: springs are rather ephemeral habitats. Another widely acknowledged idea is that the gene flow among them, if at all present, is very low. In the rich literature covering the phylogeny and population genetic structure and gene flow of the spring fauna, the gastropods are well represented [49,57,58,62-71]. Most of the studies point to low levels of gene flow and high levels of
endemism in spring snails, but on the other hand, some of the species the studies deal with are rather widespread, with much gene flow among their populations [57,58,62,63,67,71].

For long the one-species-at-one-locality view was widely accepted concerning spring-dwelling hydrobioids, like *Bythinella* [1,49]. This argument assumed that: (i) closely phylogenetically related snails have most probably the same or almost the same ecological niche; (ii) there is little or no spatial differentiation inside small and simple habitats like springs. Neither argument need be true. Firstly, the ecological niches of closely related species do not necessarily overlap. Secondly, even the smallest spring may contain more than one microhabitat. On the other hand, one can assume that two species whose ecological niches overlap will coexist if their densities are limited not by their competition, but some other factors (e.g. parasites). Falniowski et al., found an evident case of sympatric occurrence of two *Bythinella* species in Romania: striking morphological differences coupled with the molecular ones [40,49]. Two molecularly distinct sympatric taxa of *Bythinella* were found also in Greece and morphologically different - in Slovenia [24,26,72].

**Isolation VS Gene Flow**

The available data on the molecular genetics of the stygobiont gastropod did not confirm either the complete isolation of populations or their stability and longevity [1,26,57,58,65,70,73]. For inhabitants of such miniature and changeable habitats survival must depend on dynamic processes of colonization and recolonization events coupled with short- or (in some cases) long-distance dispersal [74,75].

The several cases of presumed long-distance passive transportation (the sea included), reflected by genetic identity/similarity, were reported in several studies [26,74,75]. They confirm that passive dispersal, most probably by birds is an important factor that moulds the geographical distribution of haplotypes [76-79]. There are no observations on the bird transport of spring snails, but the data on snails transported by birds are numerous. Lyell mentioned the possibility of transport by waterfowl to distant islands for a land snail *Saccinea putris* through the attachment of eggs to feathers and Darwin observed ducks emerging from a pond with duckweed adhering to their backs [80,81]. For the hydrobioids, Bondesen & Kaiser mentioned a possibility for *Potamopyrgus antipodarum* and other prosobranchs of attaching themselves to feathers by trapping them between operculum and shell. Cadée observed living *Perringia ulvae* emerging from faeces of the shelduck, *Tadorna tadorna*, indicating that they survived the passage of the digestive tract [82-84]. The numbers of *Perringia* observed alive in the shelduck’s faeces were considerable: three droppings contained in total 140 living specimens and 960 empty and/or broken *Perringia* shells [83].

Animals adapted to cave environments are also thought to be highly geographically isolated because of their limited dispersal ability, resulting from limited physiological tolerances and, especially in the case of snails, physical limitations of their locomotion [85,86]. However, this isolation is not obvious. In fact, there are many subterranean habitats which are not caves, but also harbour eyeless, depigmented animals, especially unconsolidated sediments bordering and underly- ing streams and rivers. They are parts of the interstitial habitat, neither rare nor discontinuous, making thus possible migration between caves [87-91].

And, last but not least, nearly all the debate concerns the isolation as the only factor shaping speciation in stygobiont snails. This need not to be justified: at first, even in isolation, speciation need not to proceed, in a habitat which is stable but not necessarily favourable for an animal, there would be either no mutation or each mutation would be eliminated by strong selection. Extremely low level of genetic diversity in stygobiont *Pontobelgrandiella* throughout Bulgaria may be an example. On the other hand, sympatric speciation must be also considered in the debate on the stygobiont species and speciation [92-94].

**Concluding Remarks**

In hydrobioid snails simple plesiomorphic shells, whose variability is high and overlapping between the taxa, coupled with the soft part morphology and anatomy, simplified because of miniaturisation, and unified, as a result of necessary adaptations (which often arose parallel, thus represent homoplasies) to the life in freshwater habitats (osmoregulation, internal fertilization and eggs rich in yolk and with the capsules), drastically decrease the usefulness of morphology in taxonomy, also at the species level. Cohesive species concept, most appropriate here, often cannot be applied since we know too little about those snails. It is it necessary to use molecular markers in species discrimination, although this should be done carefully, considering genetic distances calibrated at low taxonomic level. Morphostatic mode of evolution seems to dominate in many hydrobioid taxa. There is common believe in crucial place of isolation as a factor shaping speciation in long-lasting and completely isolated habitats. However, springs are rather ephemeral habitats, in fact, and there is a net of interstitial habitats which may be used as the ways of expansion. Also the actual genetic structure of (Meta) populations of those stygobiont gastropods (not necessarily low intrapopulation polymorphism, not always low levels of gene flow) contradicts the crucial role of isolation.

**References**


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