

# Biological diversity of leeches (Clitellata: Hirudinida) based on characteristics of the karyotype

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**ABSTRACT.** The majority of studies on leeches are related to: internal and external morphology. Fewer molecular research projects focus on molecular studies and karyology. The latter are needed to explain the evolutionary and systematic issues. Karyotypes for 22 species of Hirudinida have hitherto been determined, including: 8 species belonging to the Glossiphoniidae family, 3 species of Piscicolidae, 7 of Erpobdellidae, 1 of Haemopidae and 3 of Hirudinidae. The chromosome number vary among individual groups of leeches. Within Glossiphoniidae the chromosome diploid number ranges from 14 to 32, in Piscicolidae from 20 to 32, in Erpobdelliformes from 16 to 22, and in Hirudiniformes from 24 to 28. The karyological analyses were used to show phylogenetic relations between main groups of Hirudinida, and the diploid number of 16 was suggested to be a primitive value. This number tends to increase as the evolution progresses. The phylogeny scheme of leeches proposed by Mann shows the Glossiphoniidae as primitive to the Piscicolidae, and Hirudinidae as giving rise to the Haemopidae and Erpobdellidae. Those hypotheses are herewith confronted with morphological, molecular, karyological, ecological and behavioral data.

**Key words:** Hirudinida, leeches, karyology

## Cytogenetic analyses vs. other research methods in leech investigations

As an object of research, leeches (Hirudinida) are very interesting, but at the same time difficult to study. Those difficulties include:

- objective difficulties resulting from possible processes of cryptospeciation [1, 2],
- subjective difficulties resulting from the return to examination of external non-functional (non-variational, e.g., [3]) morphology.

Therefore, the taxonomy of leeches, which depends on the description area (increasingly depends on the area of phylogenetic reconstruction) presents numerous problems to researchers.

Consequently, the leech studies, as well as studies of other groups, is conducted along the following paths: morphology on various organisation levels, molecular research, and karyology. It appears that the potential of internal and external morphology is not yet exhausted as, in these terms, the main groups of leeches are not explored equally well. Other studies, of equal importance, which have

yielded phylogenetic results, except on their own, are related to spermatogenesis [4], and recently to the ultrastructure of an ovary and oogenesis [5, 6]. First of all, the comparative anatomy of Glossiphoniidae is still poorly explored. The functional morphology of Hirudinida has yet to be explored and described.

Siddall et al. [2] studied higher-level relations within Clitellata (employing taxonomically extended samples, over 100 species of annelids, and using only molecular data, i.e., nuclear 18S rDNA and mitochondrial cytochrome *c* oxidase subunit I gene sequences). On basis of these results, Siddall et al. [2] proposed that Clitellata should be synonymised with Oligochaeta, and also supported the hypothesis of common origin of Lumbriculida, Hirudinida, Branchiobdellida and Acanthobdellida (previously proposed by Brinkhurst and Gelder [7], Purschke et al. [8], Brinkhurst [9], Siddall and Burreson [10, 11]).

Phylogenetic assessment based on morphological, behavioural and molecular data has provided ample grounds for understanding the evolutionary history of leeches [3, 10–29].

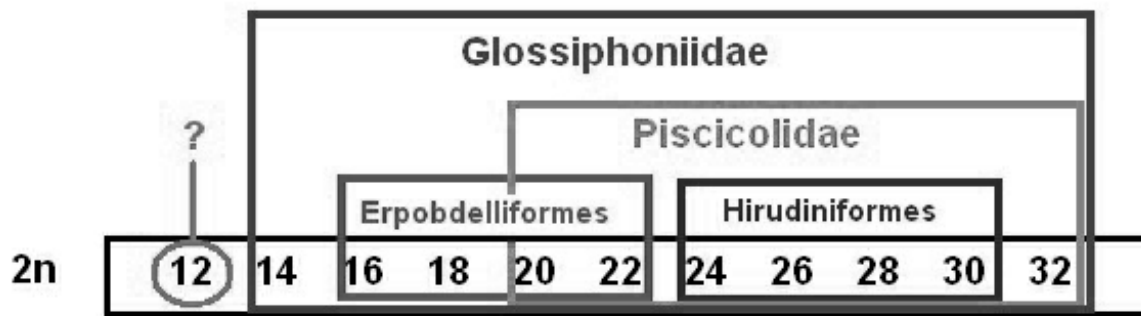


Fig. 1. The scheme presenting ranges of diploid chromosome numbers within Hirudinida

Karyological studies apply to the number of chromosomes in cell nuclei of particular leech species. They are an initial research tool in genetic, evolutionary, systematic and ecological analysis of leeches. As it turns out, the application of only general morphological and biological data is insufficient to provide an explanation of those problems [30]. It is relatively easy to study the number of chromosomes; for this reason they have been regarded as highly useful in the analysis of leech evolution [17, 31].

### The current state of investigations of the karyology of leeches

Karyological studies of leeches are dated on beginnings of 20<sup>th</sup> century. So far, the number of chromosomes has been determined for 22 leech species in 15 genera belonging to 5 families of Hirudinida (Table 1). It is relatively small amount of data, and it is partly result of technical problems, that are first of all associated with small size of chromosomes, their tendency to clump at metaphase of cell divisions and difficulties of isolation of the gonads [32]. Studies of karyotypes included freshwater and marine leech species, haematophagous and carnivorous leech species. If we talk about area of occurrence, the examined members came from North America (mainly from northern United States and Alberta, Canada) and Europe (mainly from England). The particular groups of leeches characterize with various degree of differentiation of chromosome number (Figure 1).

#### Glossiphoniidae

Within Glossiphoniidae, numbers of chromosomes were determined for 8 species. So far, it is the best investigated leech family considering the number of chromosomes.

Karyological studies have helped to resolve problems with the systematic of the genus *Theromyzon*, in which species were determined based on the body colour and the number of annuli between the gonopores, as well as on the internal morphology of the reproductive and digestive systems [33]. *T. tessulatum* is a commonly found species, typical of Holarctic, Neotropic and Ethiopian regions, while *T. rude* (its external features are highly characteristic) is typical of North America [34]. Sawyer [35] suggested, that the *Theromyzon* species from North America might be variants of *T. tessulatum*. However, analyses of karyotype support the distinction between these species, because *T. tessulatum* has  $2n=16$ , and *T. rude* has  $2n=14$  [13].

In genus *Glossiphonia* we can notice large differentiation of diploid chromosome numbers. Diploid number of 16 which was recorded in *Alboglossiphonia heteroclita* [13], supports validity of transfer of these species to the genus *Alboglossiphonia* belonging to the subfamily of Haementeriinae [18] (previously subgenus belonging to the genus of *Glossiphonia* [36]), because the value of chromosome number distinct from diploid number of 26 and 28 in *G. complanata* and *G. concolor*, respectively [13].

*Glossiphonia complanata* is a species which is common throughout Canada, the northern United States and across Europe. Diagnostic features of the species include: colour pattern and number of eyes – which are of considerable variability. Examination of the number of chromosomes in *G. complanata* from England and from the province of Alberta have helped to verify the hypothesis that it is a cosmopolitan species, found both in North America and in Europe [34].

The highest diploid chromosome number within Glossiphoniidae has noticed in *Hemiclepsis mar-*

Table 1. Haploid and diploid chromosomes number of Hirudinida (Singhal et al. 1986, Davies and Singhal 1987, Vitturi et al. 2002, Utevsky et al. 2007, Animal Genome Size Database 2008)

Species	n	2n	Authors
<b>Glossiphoniidae</b>			
<i>Placobdella papillifera</i> (Verrill, 1872)	12	24	Davies and Singhal 1987
<i>Theromyzon rude</i> (Baird, 1863)	7	14	Wendrowsky 1928
<i>T. tessulatum</i> (O.F. Müller, 1774)	—	16	Wendrowsky 1928
<i>Alboglossiphonia heteroclita</i> (L., 1761)	8	16	Wendrowsky 1928
<i>Glossiphonia complanata</i> (L., 1758)	13	26	Wendrowsky 1928
<i>G. concolor</i> (Apathy, 1888)	14	28	Wendrowsky 1928
<i>Hemiclepsis marginata</i> (O.F. Müller, 1774)	16	32	Wendrowsky 1928
<i>Helobdella robusta</i> Shankland, Bissen et Weisllat, 1992	9	18	Animal Genome Size Database 2008
<b>Piscicolidae</b>			
<i>Piscicola geometra</i> (L., 1758)	16	—	Jørgensen 1913
<i>Branchellion torpedinis</i> Savigny, 1822	—	12	Perez 1907
<i>Pontobdella muricata</i> (L., 1758)	10	20	Mancino and Puccinelli 1964
<b>Erpobdellidae</b>			
<i>Dina lineata</i> (O.F. Müller, 1774)	9	18	Wendrowsky 1928
<i>Erpobdella octoculata</i> (L., 1758)	8	16	Jørgensen 1908
<i>E. punctata</i> (Leidy, 1870)	8	16	Singhal and al. 1986
<i>E. testacea</i> (Savigny, 1822)	11	22	Puccinelli and Mancino 1965, 1966
<i>Nephelopsis obscura</i> Verrill, 1872	11	22	Singhal and al. 1986
<i>Trocheta subviridis</i> Dutrochet, 1817	11	22	Puccinelli and Mancino 1968
<i>T. bykowskii</i> Gedroyc, 1913	11	22	Puccinelli and Mancino 1968
<b>Haemopidae</b>			
<i>Haemopsis sanguisuga</i> (L., 1758)	13	26	Wendrowsky 1928, Vitturi et al. 2002
<b>Hirudinidae</b>			
<i>Hirudo medicinalis</i> (L., 1758)	14	—	Utevsky et al. 2007
<i>H. verbana</i> Carena, 1820	13	—	Utevsky et al. 2007
<i>H. orientalis</i> Utevsky et Trontelj, 2005	12	—	Utevsky et al. 2007

*ginata*, which has  $2n=32$  [13]. Other species belonging to this family which karyotypes are known are: *Placobdella papillifera* with  $2n=24$  [34] and *Helobdella robusta* with  $2n=18$  [37].

#### Piscicolidae

Diploid numbers of chromosome were determined only for three species of Piscicolidae family. The lowest value,  $2n=12$ , was recorded in *Branchellion torpedinis* [38]. However, such low value seems to be doubtful and require confirmation. In *Piscicola geometra* only haploid chromosome number was determined, and it was  $2n=16$  [39], thought, basing on this data we can suppose that diploid number for this species is 32. The third species is *Pontobdella muricata* with  $2n=20$  [31].

#### Erpobdellidae

Respectively small differentiation of diploid chromosome number has been noticed in family of Erpobdellidae. Within the genus *Erpobdella* the same diploid number,  $2n=16$ , appears in two

species: North American *Erpobdella punctata* [32] and European *E. octoculata* [40]. But another European representative of the genus, *E. testacea*, has  $2n=22$  [41], this value has been recorded also in other analysed, with regard on karyotype, species belonging to Erpobdellidae, namely *Nephelopsis obscura* [32], *Trocheta subviridis* and *T. bykowskii* [42]. Middle value,  $2n=18$ , was recorded in *Dina lineata* [13].

#### Haemopidae

Diploid number of chromosomes was determined for only one species of the Haemopidae family, *Haemopsis sanguisuga*, which has  $2n=26$  [13, 43].

#### Hirudinidae

In 2007 Utevsky et al. [44] determined numbers of chromosomes for three species of genus *Hirudo* for the first time. *Hirudo medicinalis* has  $n=14$ , *H. verbana*,  $n=13$ , and *H. orientalis*,  $n=12$ .

### Phylogeny of leeches based on characters of karyotype

In 1961 Mann [17] proposed a scheme of leech phylogenesis, according to which the primordial Oligochaeta gave rise to “Rhynchobdellida”, Arhynchobdellida and Acanthobdellida. Within “Rhynchobdellida”, the family Glossiphoniidae is regarded as primitive in relation to Piscicolidae, whereas among Arhynchobdellida, Hirudinidae gave rise to land jawed leeches (Haemadipsidae) and Erpobdellidae. Mann [17] proposal that Glossiphoniidae were more primitive than Piscicolidae is based on three-annulate somites and, what is very significant, a lower diploid number of chromosomes in representatives of the former family. However, the argument based on the number of chromosomes does not substantiate the primitive nature of Glossiphoniidae, because – according to studies – although *T. tessulatum* and *A. heteroclita* have a diploid number  $2n=16$ , *G. complanata* has diploid number  $2n=26$ , and *G. concolor*  $2n=28$ , which are higher than the diploid number of chromosomes found for *Pontobdella muricata* ( $2n=20$ ) and *Branchellion torpedinis* ( $2n=12$ ) [32]. Morphological and molecular examinations [24, 45] have clearly shown that the phylogenetic paths of Glossiphoniidae and Hirudiniformes diverged relatively early. The fundamental – it might seem, irreversible – “synapomorphy” of the proboscis in “Rhynchobdellida”, has proved to be an analogy or homoplasy. In terms of phylogenesis, Piscicolidae do not have anything in common with Glossiphoniidae and are paraphyletic in relation to the sister group of Hirudiniformes and Erpobdelliformes. Consequently, another feature of Glossiphoniidae and Piscicolidae is probably homoplasies. From an ecological perspective, parasites developed from free-living forms. Piscicolidae (fish parasites) developed from macrophagic forms of Glossiphoniidae [32]. The hypothesis is contrary to the findings of morphological and molecular studies [24, 45] because, as has been stated above, Piscicolidae are paraphyletic in relation to Hirudinidae and do not have anything in common with Glossiphoniidae.

Mann [17] has not presented any convincing evidence to substantiate another hypothesis, namely, that within Arhynchobdellida the jawed forms are primitive, while the jawless and carnivorous forms developed from them as secondary forms. Singhal et al. [32] consider it more probable that the jawed forms, which developed from free-living predatory

forms, such as Erpobdellidae, specialised. In this case annulation cannot give a confirmation, because within Arhynchobdellida it is usually 5 annuli per segment. The number of chromosomes, which in Erpobdellidae is  $2n=16-22$ , in Haemopidae (*Haemopsis sanguisuga*)  $2n=26$ , and in Hirudinidae  $2n=24-28$ , supports the supposition, that Erpobdelliformes are primitive group to Hirudiniformes. The molecular studies conducted so far indicate that Hirudiniformes are a sister group in relation to Erpobdelliformes [2, 27]. According to Borda and Siddall [27], the leech’s ancestor lived in freshwater, fed on blood and had jaws while subsequent forms acquired the proboscis.

So far, studies conducted that the number  $2n=16$  is the “primitive” diploid number of chromosomes in Hirudinida. The suggestion has been corroborated by the findings of a study into the parasitic Oligochaeta *Branchiobdella astaci* and *Acanthobdella peledina* (Acanthobdellidae) – each of them with this diploid number of chromosomes [13]. Harant and Grasse [46] suppose that Hirudinida and Oligochaeta have a common ancestor or that Hirudinida originate from Oligochaeta. The latter option is more probable, although when we talk about karyotypes, the diploid number of chromosomes is known only for one of the Branchiobdellida species (*B. astaci*), and it is not certain whether it is representative of the entire group.

According to Borda and Siddall [27, 28], the ancestral leech was related to the existing erpobdellids (or piscicolids), therefore it was adapted to the fresh-water environment. That aquatic “protoleech” laid hard-shell cocoons on a solid substrate (smooth stones, etc.); this type of behaviour is evident in aquatic erpobdellids and has been shown in the “primitive leech” *Acanthobdella peledina* [18, 25, 26, 47].

Despite a limited number of observations, it may be supposed that the evolution of chromosomes within genera resulted in differentiation of shapes, which is more apparent in Hirudinida than in Oligochaeta and Polychaeta [32]. To fully understand the evolution of chromosomes of Hirudinida, it is necessary to conduct more extensive research on a greater number of species.

### Perspectives of karyological studies of leeches

It is necessary to re-investigate the known numbers of chromosomes and examine the karyotypes

of a larger number of species, from all the representative groups. Also it is necessary to juxtapose the data with morphogenesis [47] and functional morphology in light of the leech body form model by Epstein and Bielecki [19, 21].

Apart from the determination of the haploid and diploid numbers of chromosomes, another significant aspect of karyological research is the chromosome morphology, i.e., determination of chromosome category in terms of the long and short arm ratio, occurrence in chromosomes of specific sequences: NOR, telomeric sequences and others. The data obtained in the course of studies into the number and morphology of chromosomes would probably provide information on the possible existence of polyploidisation in leeches and complement the picture of phylogenetic relationships between groups of leeches, as well as between leeches and other Annelides. The first of such data has already been presented [43].

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