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Phylogenetic analysis of the *Baikalodrilus* species flock (Annelida: Clitellata: Naididae), an endemic genus to Lake Baikal (Russia)

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Lake Baikal is populated by an endemic genus of oligochaetes (*Baikalodrilus*), which currently comprises 24 morphospecies. The genus can be considered as a 'species flock'. However, the validity of many species is questionable: the great similarity in their description and the lack of unequivocal diagnostic characters often lead species identification to an impasse. In order to clarify the systematics of this genus, we analysed two nuclear and two mitochondrial DNA markers of 40 *Baikalodrilus* specimens. DNA and morphological approaches are mostly congruent in suggesting ten candidate species, although two additional species are suspected. A reassessment of the taxonomic value of the morphological characteristics of *Baikalodrilus* suggests that there are few that can be used as distinctive, specific criteria in the genus. The association between candidate and nominal species remains problematic, except for three species identified prior to molecular analyses. ***Baikalodrilus trituberculum* sp. nov.** is described. Phylogenetic inferences suggests that the earliest split in *Baikalodrilus* and the time of divergence of most lineages corresponding to species are consistent with the hypothesis of a general rearrangement of the Baikal fauna, following major environmental changes due to a general cooling in the Early Pleistocene.

KEYWORDS: 16S – *COI* – H3 – ITS – phylogeny – systematics – taxonomy.

INTRODUCTION

> *'The whole subject of endemic speciation has to be regarded with some caution, however, as many systematists readily fall into the trap of deliberately looking for minute differences between specimens from localities known to contain endemic species and specimens from other, less exciting localities.'* (Brinkhurst, 1971: 110, 115).

Lake Baikal is the deepest (1642 m), the most voluminous (23 000 km^3) and the oldest (25–30 Mya) freshwater lake in the world, with a unique environment including ultra-oligotrophic and well-oxygenated waters at all depths. (Martin, 1994; Kozhova & Izmest'eva, 1998; Sherstyankin *et al.*, 2006). The lake harbours about 190 oligochaete species, of which more than 70% are endemic (Martin, 1996; Semernoy, 2004; Martin *et al.*, 2008). Among them, the genus *Baikalodrilus* Holmquist, 1978 requires attention, because of its monophyly, endemicity and species richness, and it is one of the more characteristic faunistic elements of the oligochaete community in Lake Baikal. The genus can be considered as a 'species flock' (Coulter, 1991) or a 'core flock' (Lecointre *et al.*, 2013).

Together with *Embolocephalus* Randolph, 1892, *Quistadrilus* Brinkhurst, 1981 and *Spirosperma* Eisen, 1879, *Baikalodrilus* belongs to a group of phylogenetically related Holarctic genera (Brinkhurst,

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1991). Their species were formerly associated with the polyphyletic genus *Peloscolex* Leidy, 1851 on the basis of the papillate nature of the body wall (Brinkhurst, 1981a). They are sometimes called 'armoured' as a reference to this sheath of adhering particles over the body surface (among others Brinkhurst & Jamieson, 1971; Holmquist, 1978; Snimschikova & Timm, 1992; Timm, 1998). The genus *Baikalodrilus* comprises 24 taxa described to date (Table 1) (Martin *et al.*, 2016). From 1901 until the beginning of the 1980s, the genus was only known by five species. A sudden increase with 14 additional species (and two subspecies) described by Snimschikova (Snimschikova, 1982, 1984, 1989a, b, 1991b) followed. Snimschikova & Timm (1992) provided an identification key to all species known at that date. After this, four more species were described: three from Lake Baikal (Semernoy, 2004) and one, *B. alienus* Timm, 1998, from Lake Taimyr (Timm, 1998).

Baikalodrilus species are only found in Lake Baikal, except *B. alienus*, although that species is considered an emigrant from Lake Baikal (Timm, 1998). The genus is characterized, among others, by a unique shuttle-shaped body, probably 'organic crystal' formed by the prostatic glands in the atrium (the so-called Michaelsen's 'Gallertstab'; Michaelsen, 1933, 1935). This is a synapomorphy unknown from any other oligochaete genus (Snimschikova & Timm, 1992; Timm, 1998). The genus was erected by Holmquist (1978), revised and emended by Brinkhurst (1981a), Snimschikova *et al.* (1987) and Snimschikova & Timm (1992).

At the turn of the 1990s, various initiatives were started to stimulate international research on different aspects of the Lake Baikal environment, in the framework of BICER (Baikal International Center for Ecological Research) (Maddox, 1989). Access to Lake Baikal was made available and this offered us the unique opportunity to collect *Baikalodrilus* specimens from different localities in the three constitutive basins of the lake. However, it soon appeared that the use of this material was hampered by the difficulty of identifying the species by their morphology. Except for a few well-defined species, interspecific differences are often minute or fall into a range of variation that are suspected to be associated with the intraspecific level. In addition, some characters are often inconsistent and even overlapping between nominal species. In practice, species identification often led to an impasse, which raised the question of the relevance of some taxonomic

Table 1. List of the 24 *Baikalodrilus* species known to date, including their protonym (Dubois, 2000: 51) when relevant. Ranges of values indicate the minimal and maximal value of body size for each species (mm)

Species	Author, date	Protonym	Body size
Baikalodrilus alienus	Timm, 1998		10.0–20.0
Baikalodrilus bekmanae	(Snimschikova, 1984)	*Peloscolex bekmani*	8.0–10.0
Baikalodrilus bifidus	Snimschikova, 1989b		8.0–8.5
Baikalodrilus crassus	Snimschikova, 1989b		32.0–35.0
Baikalodrilus cristatus	(Snimschikova, 1982)	*Peloscolex cristatus*	3.0–3.5
Baikalodrilus digitatus	Holmquist, 1979		3.0–3.5
Baikalodrilus discolor	(Snimschikova, 1984)	*Peloscolex discolor*	20.0–30.0
Baikalodrilus dividus	Semernoy, 2004		3.0–4.0
Baikalodrilus exilis	(Snimschikova, 1982)	*Peloscolex exilis*	2.1–2.3
Baikalodrilus falcatus	(Snimschikova, 1982)	*Peloscolex falcatus*	7.5–7.5
Baikalodrilus inflatus	(Michaelsen, 1901)	*Peloscolex inflatus*	40.0–40.0
Baikalodrilus intermedius	Snimschikova, 1991b		2.5–4.0
Baikalodrilus kozovi	(Hrabě, 1969)	*Peloscolex kozovi*	1.2–1.2
Baikalodrilus malevici	(Čhekanovskaja, 1975)	*Peloscolex malevici*	12.0–25.0
Baikalodrilus medianus	Snimschikova, 1991b		14.0–14.0
Baikalodrilus multicrystallifer	Snimschikova, 1989a		14.0–18.0
Baikalodrilus paradoxus	(Snimschikova, 1984)	*Peloscolex paradoxus*	20.0–24.0
Baikalodrilus parilis	Semernoy, 2004		2.0–2.5
Baikalodrilus phreodriloides	(Michaelsen, 1905)	*Lycodrilus phreodriloides*	3.0–4.4
Baikalodrilus scaphoideus	Snimschikova, 1989b		14.0–16.0
Baikalodrilus solitarius	(Snimschikova, 1982)	*Peloscolex solitarius*	11.5–?
Baikalodrilus undatus	Snimschikova, 1989b		6.0–?
Baikalodrilus vicinus	Semernoy, 2004		3.0–4.0
Baikalodrilus werestschagini	(Michaelsen, 1933)	*Peloscolex werestschagini*	3.1–3.5

decisions, in particular for a few species described by Snimschikova in the 1980s.

The main aim of this study is to clarify the systematics of this genus, based on a reassessment of morphology and molecular analyses using two nuclear (ITS and H3) and two mitochondrial (*COI* and 16S) DNA markers. In addition, the 'species flock' nature of *Baikalodrilus* offers a unique opportunity to investigate the limits of the DNA barcoding gap approach to delineate species in oligochaetes. Although the use of genetic divergence for inferring species has been extensively criticized (a.o. Hickerson *et al.*, 2006; Meier *et al.*, 2006), it is recognized that local barcoding gaps may exist (Kvist, 2016). Inferring a potential DNA barcoding gap requires that (1) 'true' sister-species are compared (lower limit of interspecific divergence) and (2) specimens are representative of the full geographic range of the species (higher limit of intraspecific variation) (Meyer & Paulay, 2005). These expectations are perfectly met in the *Baikalodrilus* species flock.

The present study is based on a dataset of 40 *Baikalodrilus* specimens, hence it is probably not representative of the full taxonomic coverage. However, we feel justified in presenting this information because of the logistic difficulty in obtaining this interesting material.

MATERIAL AND METHODS

Taxa and specimens

Baikalodrilus specimens were collected between 1994 and 1996, from different localities, at various depths, in the three basins of Lake Baikal (Supporting Information, Fig. S1; Table S1). The specimens were preserved in 95% ethanol in the field. They were subsequently kept at –20 °C in the laboratory until further processing. When possible, specimens were separated into two parts: an anterior part for voucher preparation, roughly corresponding to the first 20 segments, hence including the genital region and the clitellum when mature, and a posterior part, consisting in the remaining segments, used in full or in part for DNA processing. A few specimens were too small to be dealt with in this way (*B. werestschagini*, *B. digitatus* and one *B. malevici*). These were first identified under a stereomicroscope as whole mounts in a drop of ethanol, and then used *in toto* for DNA extraction.

DNA analyses

Taxa: We analysed a dataset consisting of 46 specimens, of which 40 were *Baikalodrilus*. The remaining six specimens were included as outgroup, four of them belonging to other papillate genera formerly included in the polyphyletic genus *Peloscolex* (*Embolocephalus* and *Spirosperma*; Supporting Information, Table S1).

DNA extraction, PCR amplification and sequencing: DNA was extracted either from the entire specimen (*B. werestschagini*, *B. digitatus* and some *B. malevici*) or from a slice cut in the posterior part of the specimen. DNA extractions were mostly done according to a ChelexTM procedure (Walsh *et al.*, 1991). The QIAgen DNA Mini Kit was used for a few samples, according to the manufacturer's protocol for animal tissue. Two mitochondrial genes (*COI* and 16S rDNA) and two nuclear genes (ITS and H3) were successfully amplified and sequenced, according to the following protocols.

COI: The 658 bp fragment of the 5' end of the cytochrome oxidase *c* subunit I gene – recommended as a standard barcode fragment in animals (Hebert *et al.*, 2003) – was amplified according to Martin & Ohtaka (2008).

16S rDNA: The standard primers 16Sar-L and 16Sbr-H (Palumbi, 2002) were used for the amplification and the sequencing of a 478–483-bp long fragment of the mitochondrial 16S ribosomal DNA. The same reaction mix as for *COI* was used. The amplification profile started with an initial step of 3 min denaturation at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 45 °C and 60 s at 72 °C, with a final step of 10 min extension at 72 °C.

ITS: The DNA fragment comprising the 3' end of the 18S ribosomal RNA gene, ITS1, the 5.8S ribosomal RNA gene, ITS2 and the 5' end of the 28S ribosomal RNA gene (929–1048 bp) was amplified using primers ITS-5-F and ITS-4-R (White *et al.*, 1990) and the same protocol as the other markers with an annealing temperature of 50 °C

H3: A 328-bp fragment of the histone H3 gene was amplified using primers H3F and H3R (Brown *et al.*, 1999) with the same protocol as for ITS.

PCR products were purified using ExoSAP-IT (ThermoFisher). Purified PCR products were sequenced in both directions using the BigDye Terminator v.3.1 Cycle Sequencing kit (Life Technologies) with the primers used for amplification, and on an ABI 3130xl Genetic Analyser (LifeTechnologies).

Alignments: Alignments were made using CLUSTAL W (Thompson *et al.*, 1994) with default settings, as implemented in MEGA v.6.06 (Tamura *et al.*, 2013). ITS regions are well known to present length variability due to their propensity to accumulate insertions/deletions (indels), making assembly of a correct and aligned ITS dataset more challenging than with other sequences (Álvarez & Wendel, 2003). However, taking

into account their secondary structure may be useful for their alignment (Giudicelli *et al.*, 2017). For this reason, two additional alignments were performed for ITS, using LocARNA (Will *et al.*, 2007; Smith *et al.*, 2010; Will *et al.*, 2012) and the Q-INS-i method of MAFFT v.6.5 (Katoh & Toh, 2008), in order to assess the effects of different alignments on tree topologies and supports.

Distance analysis: Numbers of variable sites were counted using MEGA v.6.06 (Tamura *et al.*, 2013). The *COI* dataset was further analysed as it is the most variable DNA fragment among those sequenced here and because it is a standard DNA barcoding marker (Hebert *et al.*, 2003). Pairwise uncorrected p-distances among *COI* sequences were calculated within and among specimen groups and plotted as histograms using the R package APE (Paradis *et al.*, 2004).

Single-locus species delimitation: Species delimitation based on *COI* pairwise distances was performed using the automatic barcode gap discovery (ABGD) method (Puillandre *et al.*, 2012). This method detects gaps in the distribution of pairwise distances among samples and partitions the dataset in candidate species without any a priori knowledge of the species identity (*de novo* approach). Analyses were done on the basis of uncorrected p-distances, with prior maximum divergence of intraspecific diversity between 0.001 and 0.1 (10 steps) and relative gap widths of 0.5, 1.0 and 1.5.

A haploweb was constructed using ITS haplotypes to visualize both the evolutionary paths among the haplotypes found in different individuals (haplotype network) and the co-occurrence of haplotypes in heterozygous individuals (Flot *et al.*, 2010). Haplotypes that co-occur in heterozygous individuals define fields for recombination (FFRs) as defined by (Doyle, 1995) and designate groups of individuals sharing alleles, a feature that can be used to delimit species (Flot *et al.*, 2010). For this analysis, we identified the double peaks in the electropherograms and converted the sequences with ambiguity codes into two haplotypes. When more than one double peak was found per electropherogram, sequences were converted using seqPHASE (Flot, 2010) and submitted to PHASE (Stephens *et al.*, 2001; Stephens & Donnelly, 2003) to estimate the most likely phased haplotypes. Finally, the haploweb was created from the aligned set of haplotypes using the online tool HaplowebMaker (https://eeg-ebe.github.io/HaplowebMaker/) where median joining, with epsilon = 0, was chosen as algorithm to build the haplotype network (Bandelt *et al.*, 1999) and with indels as a fifth character.

Another *de novo* species delimitation analysis was performed using the generalized mixed Yule-coalescent (GMYC) model (Pons *et al.*, 2006; Fujisawa & Barraclough, 2013). This analysis is based on the phylogenetic species concept. It was done using the R package splits v.1.0–19 (Ezard *et al.*, 2009), with the single threshold option. The ultrametric strict clock tree obtained for the *COI* dataset (see below) was used as input.

Molecular phylogenies: Molecular phylogenies were inferred from seven datasets: one concatenation of the two mitochondrial fragments (*COI* and 16S), three alternative alignments of the nuclear DNA fragment (ITS) and three concatenations of the three fragments (*COI*, 16S and ITS) using the different alignments of ITS. Unique haplotypes were extracted using R package 'pegas' (Paradis, 2010). Variability at H3 was low among the *Baikalodrilus* specimens (see Results below). H3 was, therefore, only sequenced for part of the sampling and was not included in the combined datasets. Best trees using the maximum parsimony criterion (MP), and without considering indels, were searched using the parsimony ratchet (Nixon, 1999) implemented in the R package 'phangorn' (Schliep, 2011) and with 500 non-parametric bootstrap replicates. Best partition scheme and best-fit substitution models for maximum likelihood (ML) and Bayesian inference (BI) were estimated using PartitionFinder v.1.1 (Lanfear *et al.*, 2014) on the basis of one partition for 16S, one for ITS and three for *COI*, in the latter case with one partition defined for each codon position. Analyses using the ML method were conducted with 100 bootstrap replicates by the GARLI web service hosted at molecularevolution.org (Bazinet *et al.*, 2014) using GARLI v.2.01 (Zwickl, 2006). Bayesian inferences were performed with MrBayes v.3.2.6 (Ronquist *et al.*, 2012) on the CIPRES Science Gateway (Miller *et al.*, 2010). Two parallel runs, with four chains each, were executed for ten million generations, with unlinked nucleotide substitution parameters for each data partition and the non-clock model. Every 1000th generation was sampled, and the first 25% of the trees were discarded ('burn-in'). In addition, strict and relaxed-clock models were applied to two datasets: *COI* and the concatenation of the three markers (with ITS aligned using LocARNA). Approximate time-calibration was performed on the basis of the *COI* dataset using a clock rate prior 'clockratepr = normal (0.014,0.005)'. This approximation of 1.4 ± 0.5% substitution per million years (Myr) was based on the rate of sequence divergence reported for the genus *Alpheus* (Decapoda, Caridea) (Knowlton & Weigt, 1998) in the absence of calibration specific to annelids (see: Martin *et al.*, 2010). Convergence of the runs onto the stationary distribution were checked

(average standard deviation of split frequencies < 0.01). Burn-in was also checked (likelihood of the cold chain stops to increase and randomly fluctuates around a stable value). Convergence diagnostics of the samples of the parameter values and of the branch and node parameters were also verified (potential scale reduction factor, PSRF between 1.00 and 1.02).

Morphological study

The anterior parts of the worms were dissected after hardening the tissues in 7% formalin overnight, stained with Mayer's paracarmine, and mounted in Canada balsam according to Timm & Martin (2015) for identification. The specimens were observed with a Leica stereomicroscope equipped with differential interference contrast. Voucher material was deposited in the collection of the Royal Belgian Institute of Natural Sciences (RBINS), Brussels (Supporting Information, Table S1).

Using the consensus molecular phylogeny obtained with concatenated genes, we compared sister specimens from terminal branches to deeper nodes and explored to what extent supported clades can be morphologically differentiated. *Baikalodrilus* species are usually discriminated according to features related to body size, setae, papillae and cutaneous cover, setal tubercles [= glandular tubercles *sensu* Hrabě (1982)] and male genitalia (including atrial 'crystals') (Snimschikova & Timm, 1992). These characters were investigated in more detail, and their different possible states were identified (Table 2), except for male genitalia due to their basic morphological uniformity (see also Discussion) and scarcity of mature specimens. Comparison of voucher specimens and management of descriptive data was facilitated by the software Xper2 v.2.3.2 (Ung *et al.*, 2010).

Although Holmquist (1978, 1979) carefully revised the genus '*Peloscolex*' the use of some generic morphological and anatomical terms remained inconsistent in the subsequent literature, which is confusing when assessing morphological features. 'Armour', 'papillae' and 'setal/glandular tubercles' are such terms that require clarification.

Armour: According to most authors (a.o. Brinkhurst & Jamieson, 1971: 448; Holmquist, 1978:196; Hrabě, 1969; Timm, 1998: 25, fig. 13), the 'armour' refers to the cutaneous cover of foreign particles, glued together and to the cuticle by cutaneous secretion. Yet, other authors regard the thickened epidermis itself, bearing sensory and secretory papillae, as the 'armour' (e.g. Snimschikova, 1991a: 221; Snimschikova & Timm, 1992: 56). Moreover, thickening of the epidermis is often due to the natural contraction of the body surface rather than a cellular thickening *sensu stricto* (see Papillae below). To add further confusion, some authors (e.g. Snimschikova & Timm, 1992) often confuse the epidermis and the cuticle, the latter being a non-living external layer secreted by the epidermis (Brusca & Brusca, 2003: 54). In this study, we use the term 'armour' in its narrow sense, i.e. a 'cutaneous cover of foreign particles, glued together and to the cuticle by cutaneous secretion.'

Papillae: Two kinds of papillae can be distinguished: 'Hülsenpapillen' and 'Sinnespapillen', following the German terminology introduced by Michaelsen (1903: 199).

'Hülsenpapillen' (Fig. 1A–C, E) is a cover of foreign particles glued together and agglomerated, giving the body surface a papillate look. These papillae may form irregular bodies, more or less scattered, or discrete bodies oval to leaf-like and densely set in a rhomboidal pattern. Originally, 'Hülsenpapillen' was defined as: '… an outer, probably chitinous sleeve, that is densely covered with more or less numerous and regular ringlets of granular, oval or short and thick leaf-shaped papillae…' ['… eine äussere, wahrscheinlich chitinige Hülse ab, die in mehr oder weniger zahlreichen und regelmässigen Ringeln dicht mit körneligen, ovalen oder kurz und dick blattförmigen Papillen...] (Michaelsen, 1903: 199). The papillae can be induced by external protrusions of epithelial cells, giving the epidermis a prickly surface, or by special structures acting as possible inductors of the aggregates (peg-like protrusions from the cuticle, slight to finger-like protrusions of epidermal tissue).

'Sinnespapillen' (Fig. 1D–F) are true papillae, i.e. usually nipple-like protrusions from the epithelium of the body surface (Holmquist, 1978: 197). If larger in size, other tissue may be included as well. They may be covered by a secretory cover. They have a sensory function.

In *Baikalodrilus* species, the retractility of the head into the body, combined with contraction of strong longitudinal muscles of the body wall, usually gives a wrinkled appearance to the body surface in the anterior segments. When cross-sectioned in longitudinal sections, these fine transverse ringlets may suggest epidermal papillae, and hence lead to confusion and erroneous species diagnostics. This is the case for *B. inflatus* (Holmquist (1978: 199) and possibly *B. paradoxus* (see below). Snimschikova (1984) and Snimschikova & Timm (1992) used the term 'papillae' inconsistently to designate 'Hülsenpapillen' and true or assumed epidermal structures.

In addition to papillae, setal/glandular tubercles (Fig. 2A–F) ('Borstentuberkeln' of Michaelsen, 1901: 143) are present in *Baikalodrilus* on each segment, probably called so due to their close vicinity to setal bundles. They are said to be glandular in *B. inflatus*

Table 2. List of morphological characters used in this study (20 descriptors, three dependent descriptors). Character selection from Snimschikova & Timm (1992)

1. Adult body length: 1. > 4 mm, 2. < 4 mm
2. Pilosity of hair setae: 1. smooth, 2. pilose
3. Ectal tip of hair setae: 1. straight, 2. hooked
4. Number of hair setae in anteclitellar segments
5. Ectal tip of needles: 1. simple pointed, 2. bifid, 3. pectinate
6. Number of needles
7. Types of ventral setae in the same bundle: 1. one type, 2. two types
   + 8. Ectal tip of ventral setae: 1. simple pointed, 2. bifid
   Conditions for inapplicability (two types)
9. Shape of ventral setae: 1. sigmoid, 2. sickle-shaped
   + 10. location of sickle-shaped setae: 1. in all ventral segments, 2. in posterior segment
   Conditions for inapplicability (sigmoid)
11. Number of ventral setae per bundle in anteclitellar segments
12. Missing ventral setae: 1. present in all segments but XI, 2. absent in midbody (IX–XIX)
13. 'Hülsenpapillen': 1. totally absent, cuticular cover translucent, 2. present and distinct in anterior segments, only narrow strips of secretions in the posteriormost segments, 3. present all over the body, distinct, 4. present, fused in a thick armour
14. Protrusions of epidermal tissue: 1. absent, 2. roundish, 3. finger-like
    + 15. Finger-like protrusion features: 1. gradually increasing in length from ventral to dorsal side of body, 2. highest protrusion forming a longitudinal ridge along the dorsal side of body
    Conditions for inapplicability (absent, roundish)
16. Number of crystals in each atrium: 1. 0, 2. 1, 3. 2, 4. 3, 5. many
17. Development of glandular tubercles: 1. absent or not visible, 2. small, weakly developed, hardly distinguishable between 'Hülsenpapillen', 3. medium, well distinguishable between 'Hülsenpapillen', 4. large, prominent
18. Number of glandular tubercles: 1. 4 in each segment, 2. 6 in each segment
19. 'Sinnespapillen': 1. absent, 2. present
20. Retracted prostomium: 1. slightly protruding out of mouth cavity, 2. hidden in mouth cavity

and are referred to as 'glandular tubercles' by Hrabě (1982). We here adopt this latter terminology, as 'setal tubercle' may suggest glandular appendages of the setal follicles, a connection with setae that is not demonstrated yet. This point will be discussed later on.

Abbreviations used in figures

a, atrium; c, crystal; cg, cerebral ganglion; dgt, dorsal glandular tubercle; dlgt, dorso-lateral glandular tubercle; ds, dorsal seta; gt, glandular tubercle; hp, 'Hülsenpapille'; ip, inductor of 'Hülsenpapillen'; m, mouth; p, prostate; pds, proximal tip of dorsal seta; pg, pharyngeal glands; ph, pharynx; pr, prostomium; pvs, proximal tip of ventral seta; rm, retractor muscle; sg, setal gland; sp, 'Sinnespapille'; vd, vas deferens; vgt, ventral glandular tubercle; vs, ventral seta.
Segments are designated by Roman numerals.

RESULTS

DNA-based phylogeny

Sequences of *COI*, 16S, ITS and H3 are obtained for 39, 37, 33 and 24 *Baikalodrilus* specimens, respectively (Supporting Information, Table S1). The H3 dataset reveals only six variable and three parsimony-informative sites, hence the gene was not sequenced for all specimens and was not used for phylogenetic analyses. Phylogenetic inferences based on the concatenated dataset were all better resolved than those based on the mitochondrial DNA (*COI* and 16S) or on ITS taken separately, and all trees showed consistent topologies. The concatenation of the three DNA fragments (*COI*, 16S and ITS) provided good support for most deep nodes, with the exception of nodes 16 and 18 (Fig. 3). Terminal nodes are generally well-supported, with a few exceptions corresponding to two categories of specimens. One category concerns mostly genetically identical specimens, which come essentially from the same locality (nodes 12 and 13; near Upper Angara and Yarki Island; see Supporting Information, Table S1; Fig. S1). A second category pertains to specimens that are genetically well-differentiated and come from distant localities, sometimes about 200 km apart. (e.g. Frolikha River and Senogda Bay vs. Maximicha Bay, Barguzin, node 27; Chivirkuysky Gulf vs. Selenga Delta, node 25; Frolikha River vs. Senogda Bay, node 28; Supporting Information, Table S1, Fig. S1).

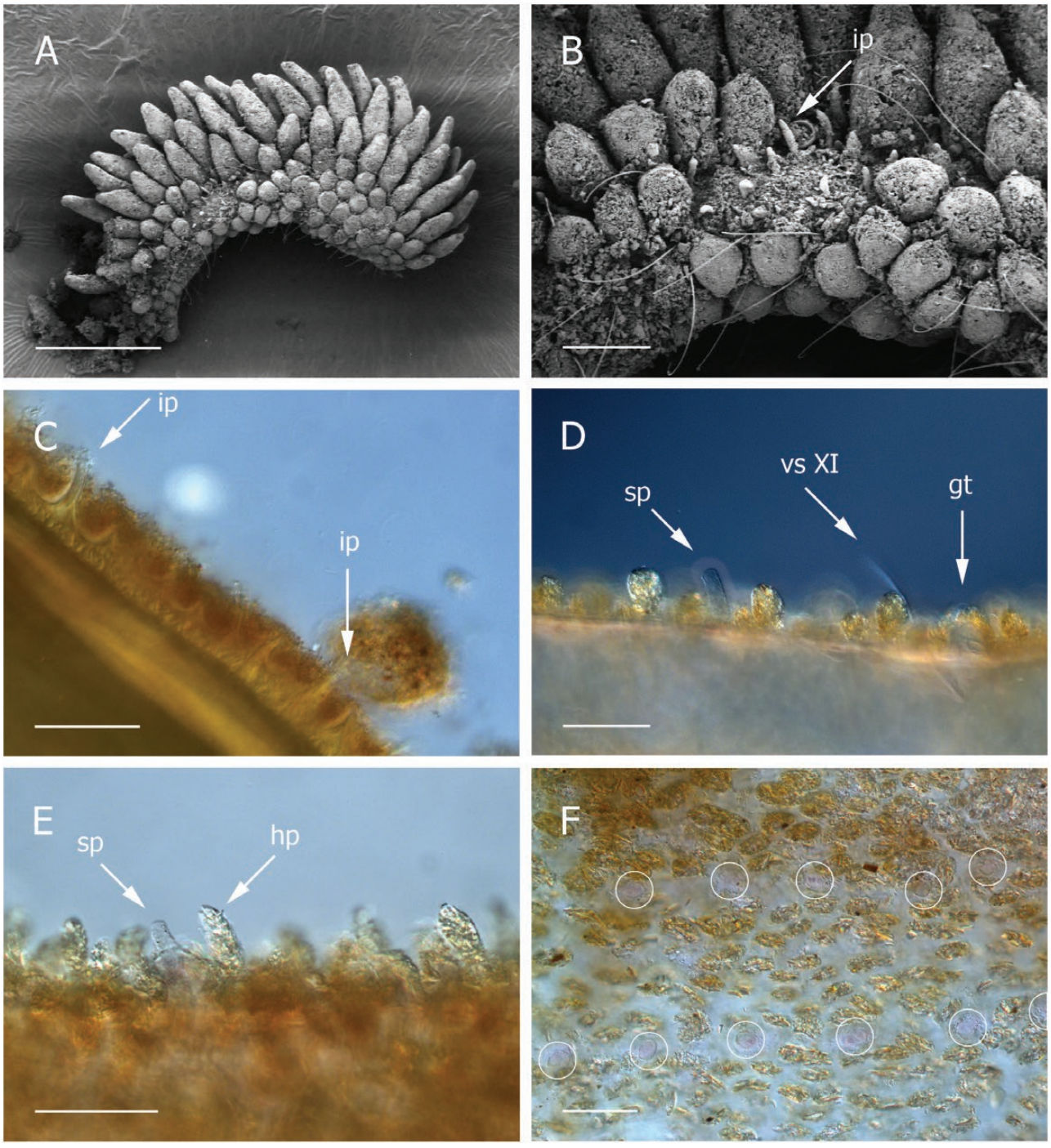


Figure 1. A–C, 'Hülsenpapillen' (for a definition, see main text) and tegumentary inductors of papillae. A, B. *B. digitatus* with some 'Hülsenpapillen' removed on part of the side of the body, showing the finger-like epidermal processes that support the elongated cover of sediment and secretions (SEM picture). C, G3, 01.036.08. D–F, 'Sinnespapillen'. D, G1, 01.076.06 (photo retouched to highlight the papilla on a lightened background). E, G5, 01.065.03. F, G5, 01.065.03: white circles have been added to highlight the two rows of papillae per segment. Group (G) and specimen codes refer to Supporting Information, Table S1. [Scale: A, B, D and E (50 μm); C and F (100 μm).]

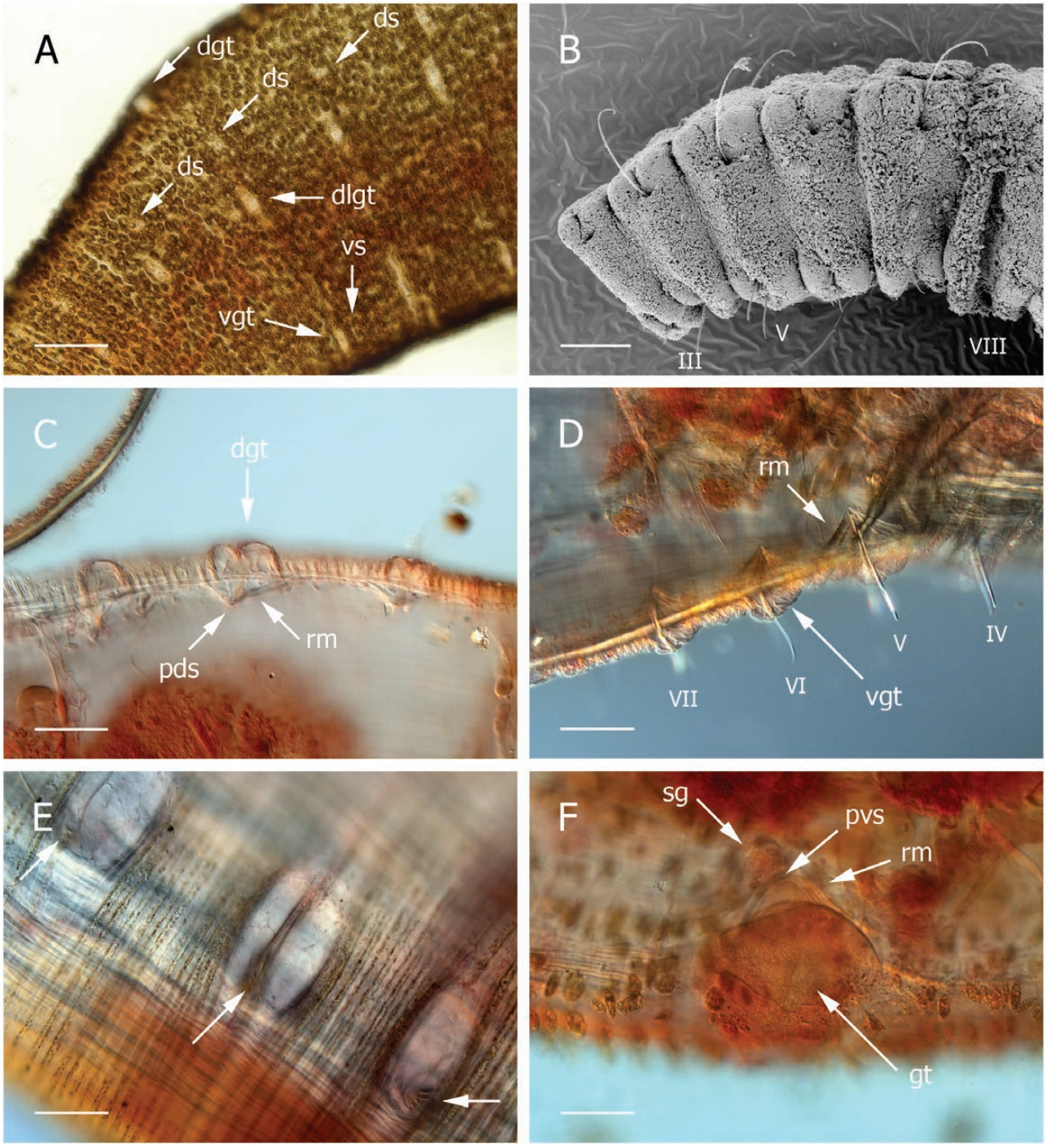


Figure 2. A–F, Glandular tubercles. A, *Baikalodrilus trituberculum*, G1, 00.346.01, picture showing the three pairs of button-like glandular tubercles characteristic of the species. B, anterior part of *B. werestschagini* showing the characteristic pad-shaped, glandular tubercles arranged in four longitudinal rows (SEM picture). C–D, G2, 01.036.03, dorsal and ventral glandular tubercles. E, G2, 01.065.09, dorsal glandular tubercles in close association with setae; arrows indicate the shaft of dorsal setae seen in more or less cross section. F, G7, 01.076.06, picture showing that glandular tubercle and ventral setal glands are unrelated structures. Group (G) and specimen codes refer to Supporting Information, Table S1. [Scale: A (200 µm); B–E (100 µm); F (50 µm).]

Trees constructed using strict and relaxed-clock models provided the same clustering and almost identical support values to the non-clock models (Supporting Information, Table S2). The time-calibrated *COI* tree based on a uniform rate of substitution suggests that the earliest split (node 1) occurred between 2.5 and 5.3 Mya and that most lineages (N = 12) correspond to candidate species that diverged between 0.9 and1.8 Mya.

Species delimitation

Distances: Pairwise p-distances measured from *COI* among *Baikalodrilus* specimens range from 0.00 to 15.25 (Fig. 4, Table 3).

ABGD: ABGD analyses suggested a partition in 1, 3, 5, 10, 12, 16, 17 or 18 different candidate species, depending on parameter settings (Fig. 3). The three first partitioning schemes suggest one, three and five candidate species, respectively, but group together specimens with clearly distinct morphology, and assigned to two different species (*B. malevici* and *B. discolor*), in one candidate species. The fourth partitioning scheme defined ten candidate species, which are supported as reciprocally monophyletic in most phylogenetic inferences. The last four partitioning schemes (12, 16, 17 and 18 hypothetical species) identified two candidate species that are not supported by any phylogenetic inference (by lack of resolution and not by conflict; see '?' in Fig. 3). Finally, the last two partitioning schemes (17 and 18 candidate species) imply in all phylogenetic inferences that the two-candidate species are paraphyletic (cf. 'x' in Fig. 3).

GMYC: The species delimitation analysis performed on the basis of the generalized mixed Yule-coalescent (GMYC) model suggested 21 species represented by one to four specimens (Fig. 3).

Haploweb: A total of 39 ITS sequences (34 specimens, including five with heterozygote positions), were used to construct the haploweb (Fig. 5). This analysis reveals 18 FFRs, i.e. groups of individuals sharing alleles (Flot *et al.*, 2010). Most of them (15/18) correspond to the partition of the GMYC analysis. Five of them correspond to the results of the ABGD analysis (Fig. 3). In general, specimens with the same ITS haplotype formed clades in the phylogenetic analysis (Fig. 3). The only two exceptions to this observation are the haplotypes shared by specimens 01.076.03, 00.346.03 and 01.076.09, and those shared by specimens 00.161.01, 00.161.04, 00.346.02, 01.065.04 and 01.076.07, which do not form any clades in the phylogenetic analyses (Figs 3, 5). Between one and three heterozygote positions are found in five specimens. Only one of these heterozygote specimens (01.065.02) shares a haplotype with other specimens (01.036.09, 01.065.03 and 01.076.02). In the phylogenetic analysis (Fig. 3), these four specimens form a clade.

Morphology

It is possible to identify four morphospecies prior to DNA analyses (Supporting Information, Table S1). Two of them are consistent with DNA-based clustering, namely *B. werestschagini* and *B. malevici* (Fig. 3). Two other morphospecies, first identified as *B. digitatus* and *B. discolor*, proved to consist of four distinct clades (Fig. 3), one of them being a new species to science (see below).

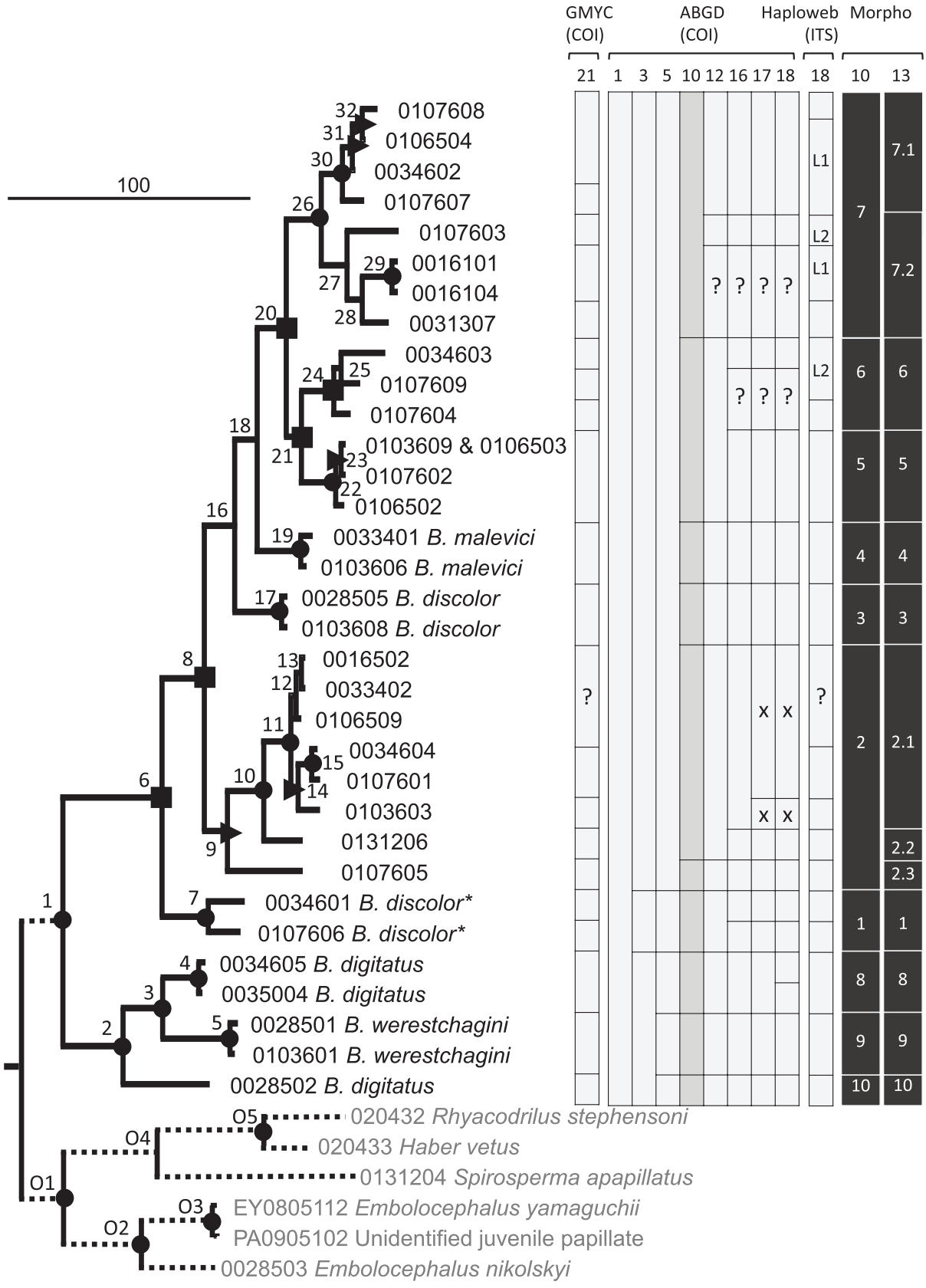
Morphological scrutiny of, and comparison between, specimens according to the phylogenetic tree structure enabled us to identify ten groups of specimens that are supported by all phylogenetic inferences, with two exceptions (Fig. 3). Group 6 is supported by BI and MP but not by ML, while group 2 is supported by BI only (Fig. 3).

Among these ten groups, three correspond to small specimens (body size shorter than 4 mm; see Snimschikova & Timm, 1992; and below), with distinctive habitus (Figs 1A, B, 2B). *Baikalodrilus werestschagini* (group 9), the so-called 'Panzeroligochät' (= armoured oligochaete; Michaelsen, 1933), is remarkable by its thick, continuous armour, with four rows of large glandular tubercles on edges on setal lines, giving its body a quadrangular shape in cross-section (Fig. 2B). Such tubercles ('Sinneshügel') are regarded as 'sensory' by Michaelsen (1933, 1935). Similarly, *B. digitatus* has distinctive, elongate 'Hülsenpapillen' covering finger-like epidermal projections of the body wall, giving the worms a resemblance to some nudibranchs (Fig. 1A, B). In spite of its distinctive species-specific habitus, *B. digitatus* appears paraphyletic, with one specimen (group 10) separated from the other two (group 8) by intervening specimens of *B. werestschagini*. Further re-examination of these specimens is unfortunately not possible as they were used *in toto* for DNA extraction.

In contrast, the seven groups corresponding to large specimens (body size longer than 4 mm) are detailed below (Table 4; Supporting Information, Table S1,). Unique features of each group are put in italics when relevant.

Group 1 (Figs 1D, 2A, 6C, F)

One immature individual (00.346.01) and one individual starting maturity (spermathecae in early stage, testes present; 01.076.06) were assigned to group



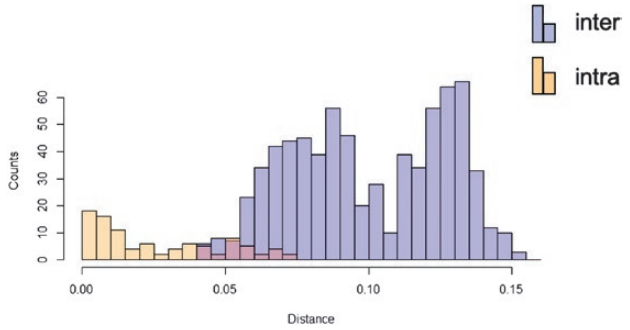


Figure 4. Distribution of pairwise uncorrected p-distances among and within *COI* sequences of the ten groups identified in the integrative taxonomical analysis (see Fig. 3).

1. The two specimens of group 1 are unique in having *three pairs of buttonhole-like glandular tubercles per segment*, on the same transversal line, one ventral, besides and in front of setae, one dorsolateral and one dorsal, dorsal setae being midway between the latter two (Figs 1D *gt*, 2A, 6F dgt, dlgt, vgt). This feature is not known in any *Baikalodrilus* species described to date. Hence, we consider this group as a new species, described below.

Group 2 (Figs 2C, D, 6D)

Eight individuals were assigned to group 2: four were immature (00.165.02, 00.346.04, 01.076.01 and 01.076.05), one had spermathecae in early stages (00.334.02) and three were sexually mature, mated specimens with spermathecae full of spermatozeugmata (00.346.04, 01.065.09 and 01.312.06). One crystal in atrial lumen, absent in 01.312.06. *Prostomium slightly protruding out of mouth cavity* (Fig. 6D, *pr*). Dorsal bundles with 1–4 pilose hair setae, with straight tip, 1–5 needles, bifid with sometimes duplicated upper tooth, teeth 4 µm long. One to four setae of both types per ventral bundle, 0–2 bifid and 0–2 simple pointed. *'Hülsenpapillen' totally absent, cuticular cover translucent* (but see Group 7 below). 'Sinnespapillen' not seen.

Remarks: Two specimens (referred to as 2.2 and 2.3 in Fig. 3), 01.312.06 and 01.076.05, slightly deviate from this general scheme. Although both have no 'Hülsenpapillen', specimen 01.312.06 has smooth hair setae and a prostomium well retracted into the mouth cavity; specimen 01.076.05 has pilose hair setae but needles have short teeth (≤ 2 µm), sometimes reduced to one to two simple notches (1 µm long).

Group 3 (Fig. 1C)

Two individuals, only one (01.036.08) available for morphological scrutiny. Specimen (post?) mature, narrow brown clitellum present, genitalia degenerating, no crystal. Prostomium well retracted into the mouth cavity. Dorsal bundles with 1–5 smooth hair setae, with straight tip, 2–3 simple pointed needles; 2–3 simple pointed setae in ventral bundles. 'Hülsenpapillen' present all over the body fragments studied, peg-like protrusion of epidermis as inductor of papillae (Fig. 1C *ip*). Two pairs of small, weakly developed glandular tubercles; dorsal glandular tubercle beside hairs; ventral tubercle hardly visible, beside setae and distinct from ectal setal glands. 'Sinnespapillen' not seen.

Group 4 (Fig. 7A)

One immature (00.334.01) and one sexually mature but unmated individual (empty spermathecae; 01.036.06) were assigned to group 4. Prostomium retracted into mouth cavity. One small crystal present in the ectal end of atrium, near ejaculatory

Figure 3. Consensus molecular phylogeny constructed using the maximum likelihood method and the concatenation of three gene fragments (*COI*, 16S and ITS). Numbers at nodes are node identifiers used in Supporting Information, Table S2. Nodes were considered as supported if posterior probabilities and bootstrap values were both higher than, or equal to 0.95 and 70, respectively, whatever the alignment method used for ITS (San Mauro & Agorreta, 2010). Node supports are illustrated with different symbols: 1, black circle are for nodes supported in all trees, whatever the phylogenetic method (Bayesian inference, maximum likelihood and maximum parsimony); 2, squares and triangles are for nodes supported in trees obtained by two or one methods, respectively; 3, unsupported nodes receive no symbols. Partitions on the right side of the figure represent (from left to right) the results of the species delimitation analyses with molecular methods, and the results of our morphological scrutiny. Question marks are for hypothetical species not supported by any phylogenetic inference (by lack of resolution, not by conflict). 'X' stands for candidate species whose acceptation as such imply paraphyly (see text). Provisional identification of species as mentioned in Supporting Information, Table S1. L1 and L2 refer to specimens attributed to two distinct candidate species, which share ITS haplotypes. Outgroup specimens are indicated with a grey lettering. Specimens identified in this study as a new *Baikalodrilus* species (*B. trituberculum*) are marked with an asterisk.

Table 3. Uncorrected pairwise p-distances (*COI*) within and between the *Baikalodrilus* groups as identified in Figure 3 (pairwise-deletion of missing data). Lower-left matrix: minimal distances between groups. Upper-right matrix: maximal distances between groups. In grey cells: distance ranges within groups

Group	1	2	2.1	2.2	2.3	3	4	5	6	7	7.1	7.2	8	9	10
1	3.50–3.50	10.18	9.27	10.03	10.18	9.57	9.57	10.94	10.18	12.01	10.64	12.01	14.13	15.25	13.07
2	7.90	0.15–7.90	-	-	-	8.21	7.60	9.42	9.57	10.33	10.33	10.03	13.37	15.25	13.37
2.1	7.90	-	0.15–2.58	4.41	7.60	7.60	7.60	9.27	9.57	10.03	9.73	10.03	12.61	12.79	11.85
2.2	9.42	-	3.80	-	7.90	8.21	7.29	9.42	9.27	10.33	10.33	9.88	12.16	11.97	12.31
2.3	8.97	-	6.84	7.90	-	7.60	7.29	9.27	8.97	9.88	9.73	9.88	13.37	15.25	13.37
3	8.97	7.14	7.14	8.21	7.60	0.30–0.30	6.23	7.45	8.21	8.81	8.66	8.81	12.92	12.79	12.16
4	8.36	6.38	6.38	6.69	6.69	5.62	0.76–0.76	6.99	7.45	8.66	8.21	8.66	13.68	12.62	12.01
5	10.03	8.36	8.36	9.12	8.66	6.84	5.93	0.61–0.91	5.32	8.05	6.99	8.05	13.22	13.93	13.98
6	9.27	7.60	7.60	8.97	8.21	7.29	5.78	4.41	2.43–4.10	8.97	7.75	8.97	14.13	14.26	14.13
7	9.57	7.90	7.90	8.51	8.36	6.99	6.38	5.62	5.17	0.30–6.38	-	-	13.53	15.08	13.68
7.1	9.57	8.36	8.66	9.42	8.36	7.75	6.38	5.62	5.17	-	0.91–2.89	6.38	12.77	15.08	12.92
7.2	10.18	7.90	7.90	8.51	8.66	6.99	6.84	6.23	6.08	-	4.71	0.30–5.32	13.53	14.59	13.68
8	13.07	10.64	10.64	11.40	12.61	12.01	12.46	11.70	12.77	11.25	11.25	11.25	0.15–1.52	7.21	7.60
9	13.53	10.94	10.94	11.25	14.74	12.01	11.70	12.77	12.92	12.61	12.77	12.61	5.93	0.30–0.82	10.33
10	13.07	10.94	10.94	12.31	13.37	12.01	11.85	13.37	13.07	12.31	12.46	12.31	6.99	9.27	-

duct. Dorsal bundles with 1–4 pilose hair setae, with straight tip, and 1–4 simple pointed needles (Fig. 7A). One to two simple pointed setae in anteclitellar, ventral bundles. *'Hülsenpapillen' present as small flakes of aggregates, without apparent tegumentary inductor, scattered on the tegument surface in anteclitellar segments, absent in postclitellar segments; only narrow strips of secretions in wrinkled epidermis in posterior segments.* 'Sinnespapillen' small, hard to spot. Two pairs of glandular tubercles, beside setal bundles.

Group 5 (Figs 1E, F, 6A, B, 7B)

Four individuals were assigned to group 5; three were available for morphological scrutiny, two mature (01.036.09 and 01.065.02), one immature (01.065.03). Specimen 01.065.02 with one large crystal in one atrium, immersed in an amorphous, granular mass; *small crystals also present* in the other atrium, in addition to a large crystal (Fig. 6A, B, C). Prostomium retracted into mouth cavity. One to three smooth hair setae per dorsal bundle, with straight ectal tip; 1–3 needles per bundle, bifid with teeth 4 µm long, or bluntly simple pointed, or simple pointed with two ectal, small notches giving a 'trifid' appearance to the needle with teeth 1 µm long (Fig. 7B). There are (1)2 setae in anterior ventral bundles, of two types, one bifid and one simple pointed in 01.065.02 and 01.065.03 but only simple pointed in 01.036.09. 'Hülsenpapillen' present as flakes of aggregates developed on small tegumentary inductors (Fig. 1E, *hp*). Two rows of 'Sinnespapillen', one just behind the transversal setal line, the other in between successive setal lines (Fig. 1E, F, *sp*). Two pairs of glandular tubercles in each segment, besides setal bundles, more developed dorsally.

Remarks: Although developed on small tegumentary inductors, 'Hülsenpapillen' do not form a sleeve surrounding the inductor, i.e. there is no internal cavity in the papillae, which usually gives them an appearance like the finger of a glove.

Group 6 (Fig. 7C, D)

Three individuals were assigned to group 6, one immature (01.076.04) and two mature (00.346.03 and 01.076.04) with degenerating genitalia; crystals absent. Prostomium retracted into the mouth cavity. One to five pilose hair setae in dorsal bundles, with straight ectal tip and 1–4 pectinate needles (Fig. 7C, D). One to five setae of both types in ventral bundles, 0–3 bifid and 0–2 simple pointed. 'Hülsenpapillen' present all over the body or totally absent with a translucent cuticular cover. Two rows

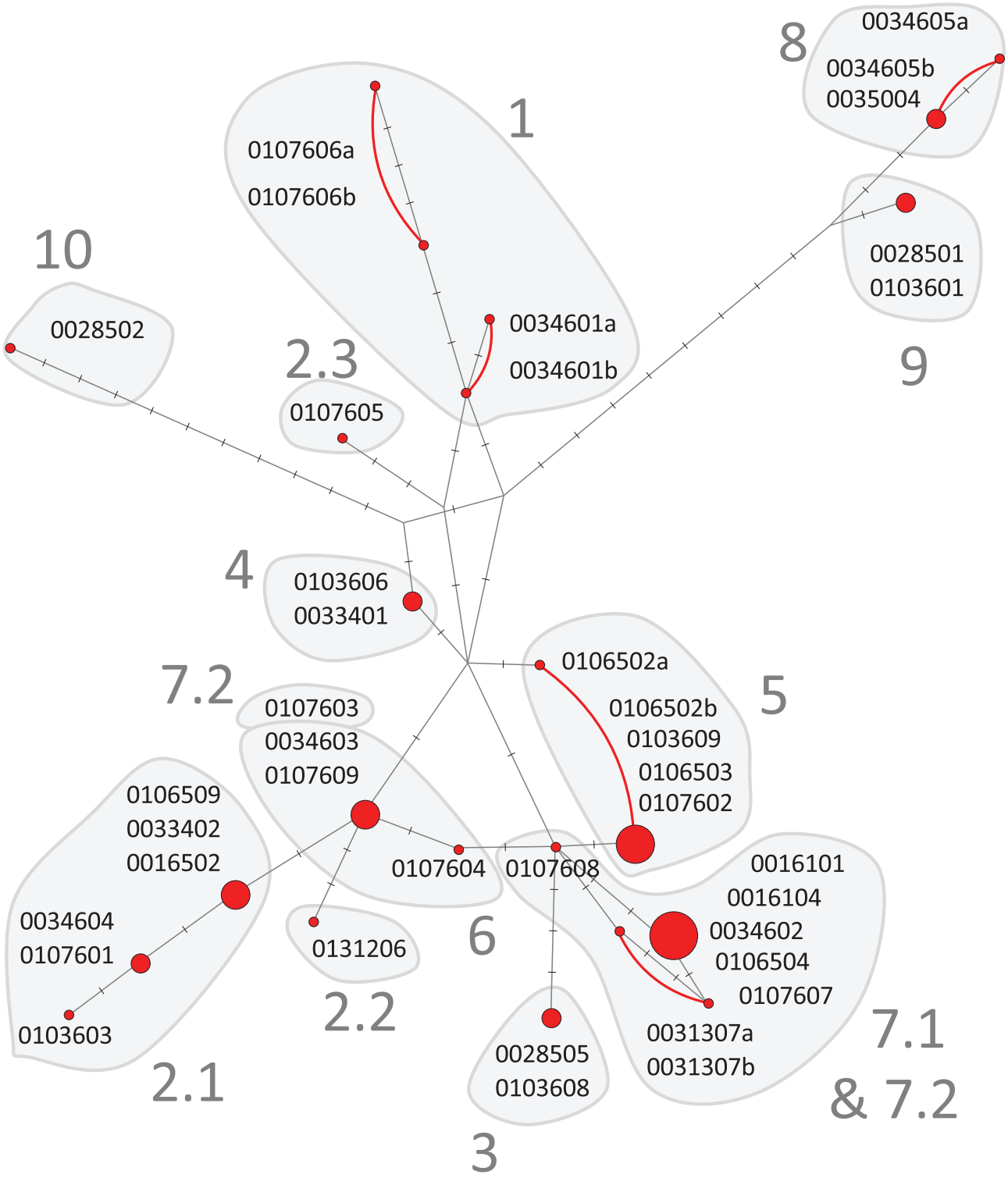


Figure 5. Haploweb built from the alignment of all ITS haplotypes using the median joining algorithm (epsilon = 0) and indels as a fifth character. Each circle represents a single haplotype whose size is proportional to the number of individuals showing this haplotype (haplotype names indicated next to each circle). The small lines drawn on the connections between two haplotypes represent the substitutions found between these haplotypes. The curves represent connections between haplotypes found in the same individual (heterozygote) whose circle are given the same colour. The grey shapes and their respective numbering represent the delimitation of species based on morphology.

Table 4. Character states for the different groups of large *Baikalodrilus* specimens identified by combining morphological and DNA sequence data. Discriminating and partially discriminating characters are highlighted in a medium and light grey background frame, respectively. Unique features of each group are shown in italics. Character selection from Snimschikova & Timm (1992) (see Table 2)

Character	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7.1	Group 7.2
Body length (adult)	longer than 4 mm	longer than 4 mm	longer than 4 mm	longer than 4 mm	longer than 4 mm	longer than 4 mm	longer than 4 mm	longer than 4 mm
Pilosity of hair setae	smooth	pilose, smooth	smooth	pilose	smooth	pilose	pilose	pilose
Ectal tip of hair setae	straight	straight	straight	straight	straight	straight	straight	straight
Number of hair setae in anteclitellar segments (Min–Max)	1–2	1–4	1–5	1–4	1–3	1–5	1–*7*	1–5
Ectal tip of needles	simple pointed	bifid	simple pointed	simple pointed	simple pointed; bifid	pectinate	pectinate	pectinate
Number of needles (Min–Max)	1–2	1–5	2–3	1–4	1–3	1–4	1–5	1–5
Types of ventral setae in the same bundle	one type	two types	one type	one type	one type;	two types	one type; two types	two types
Ectal tip of ventral setae	simple pointed	not applicable	simple pointed	simple pointed	simple pointed	not applicable	*bifid*	not applicable
Shape of ventral setae	sigmoid	sigmoid	sigmoid	sigmoid	sigmoid	sigmoid	sigmoid	sigmoid
Number of ventral setae per bundle in anteclitellar segments (Min–Max)	1–2	1–4	2–3	1–2	1–2	1–5	1–*6*	1–4
Missing ventral setae	present in all segments but XI	present in all segments but XI	present in all segments but XI	present in all segments but XI	present in all segments but XI	present in all segments but XI	present in all segments but XI	present in all segments but XI
'Hülsenpapillen'	present all over the body, distinct	totally absent, cuticular cover translucent	present all over the body, distinct	*present and distinct in anterior segments, only narrow strips of secretions in the posteriormost segments*	present all over the body, distinct	totally absent, cuticular cover translucent; present all over the body, distinct	present all over the body, distinct	present all over the body, distinct
Protrusions of epidermal tissue	absent	absent	absent	absent	absent	absent	absent	absent

Table 4. Continued

Character	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7.1	Group 7.2
Number of crystals in each atrium	?	1	?	1	*many*	?	1	1
Development of glandular tubercles	medium, well distinguishable between Hülsenpapillen	large, prominent	small, weakly developed, hardly distinguishable between Hülsenpapillen	small, weakly developed, hardly distinguishable between Hülsenpapillen	small, weakly developed, hardly distinguishable between Hülsenpapillen; medium, well distinguishable between Hülsenpapillen	large, prominent; medium, well distinguishable between Hülsenpapillen	medium, well distinguishable between Hülsenpapillen	medium, well distinguishable between Hülsenpapillen
Number of glandular tubercles	*6 in each segment*	4 in each segment	4 in each segment	4 in each segment	4 in each segment	4 in each segment	4 in each segment	4 in each segment
'Sinnespapillen'	present	absent	absent	absent; present	present	absent; present	absent; present	absent; present
Retracted prostomium	hidden in mouth cavity	*slightly out of mouth cavity*	hidden in mouth cavity	hidden in mouth cavity	hidden in mouth cavity	hidden in mouth cavity	hidden in mouth cavity	hidden in mouth cavity

of 'Sinnespapillen', one just behind the transversal setal line, the other in between successive, transversal setal lines; 'Sinnespapillen' not seen on specimen 01.076.04, which has no 'Hülsenpapillen'. Besides setal bundles, there are two pairs of glandular tubercles in each segment, equally developed dorsally and ventrally.

Remarks: The total absence of 'Hülsenpapillen' in one individual (01.076.04), while the other two specimens have 'Hülsenpapillen' covering the whole body, suggests that the armour can be occasionally shed, as Brinkhurst (1964, 1966a, 1981b) has repeatedly suggested for the genus *Peloscolex* in its earlier acceptance. To what extent the presence/absence of 'Hülsenpapillen' can be used as a specific criterion, will be dealt with in the discussion.

Group 7 (Figs 2F, 6E, 7E–F)

Eight individuals were assigned to group 7. All specimens are basically similar in morphology. However, the phylogenetic tree suggests two subgroups, one with high support in all analyses based on concatenated data (Fig. 3, node 30; Supporting Information, Table S2) and one supported in 7/12 of the analyses (Fig. 3, node 27; Supporting Information, Table S2). Subgroup 1 (7.1 in Fig. 3): two immatures (01.076.07 and 01.065.04) and two mature (00.346.02 and 01.076.08) individuals. Subgroup 2 (7.2 in Fig. 3): two immatures (00.313.07 and 01.076.03) and two mature (01.161.01 and 01.161.04) individuals. Prostomium retracted into mouth cavity (Fig. 6E, pr). One to seven pilose hair setae in dorsal bundles, with straight ectal tip, and 1–5 pectinate needles (Fig. 7E, F). One to six setae in anteclitellial ventral bundles, of two types, except specimen 01.076.07 where all setae are bifid. 'Hülsenpapillen' distinctly present all over the body. Two rows of 'Sinnespapillen', one just behind the transversal setal line, the other in between successive, transversal setal lines; 'Sinnespapillen' not seen on some individuals. Glandular tubercles well visible between 'Hülsenpapillen', present both dorsally and ventrally, besides setal bundles. One distinct crystal in each atrial lumen.

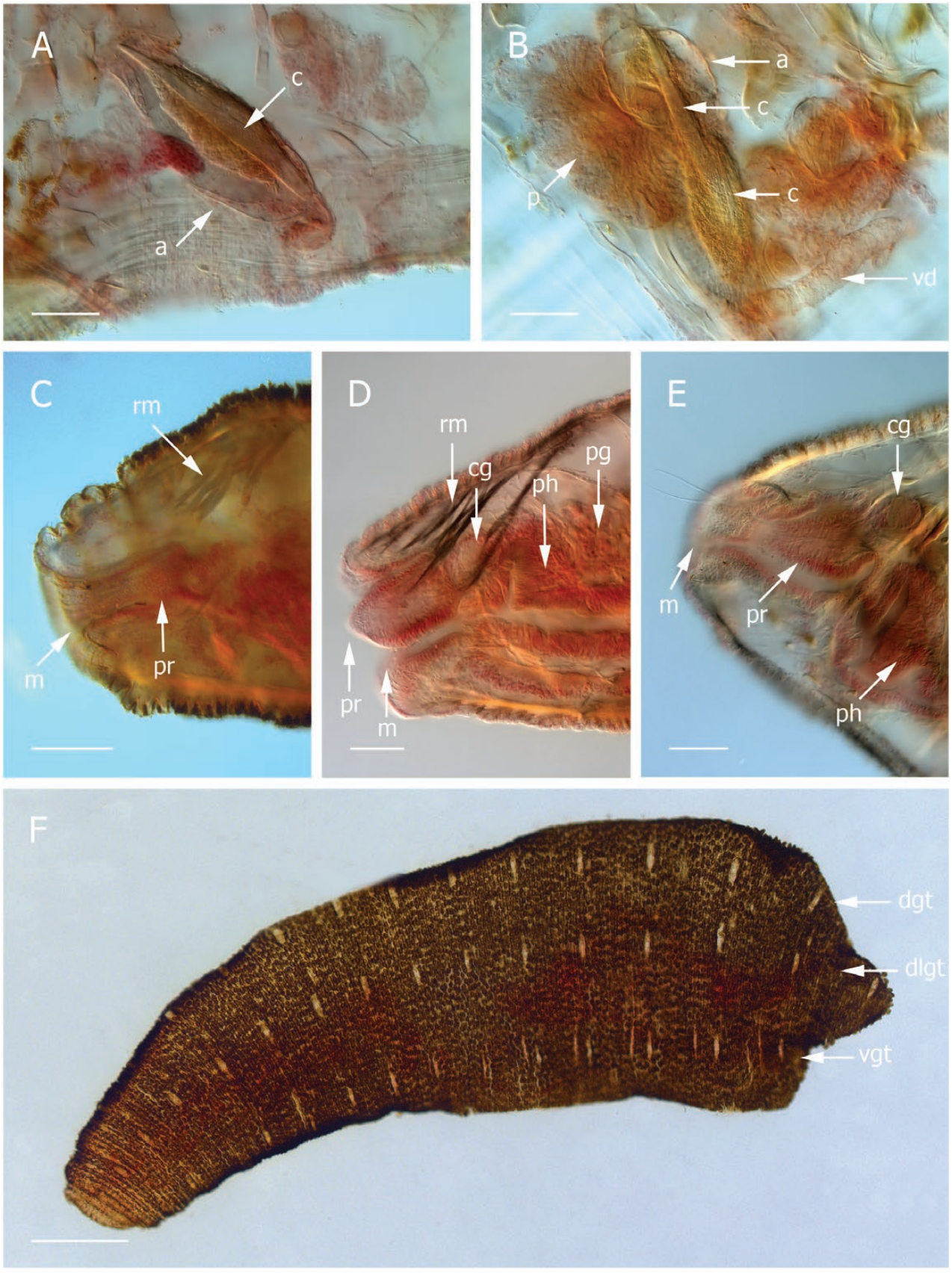
TAXONOMY

BAIKALODRILUS TRITUBERCULUM MARTIN, **SP. NOV.**

(FIGS 1D, 2A, 6C, F)

lsid: urn:lsid:zoobank.org:act:7BF41A46-DAD9-4B81-BBEA-49CA00DFD606

Holotype: RBINS 00.346.01, fragment (first 17 anterior segments) of one individual starting maturity



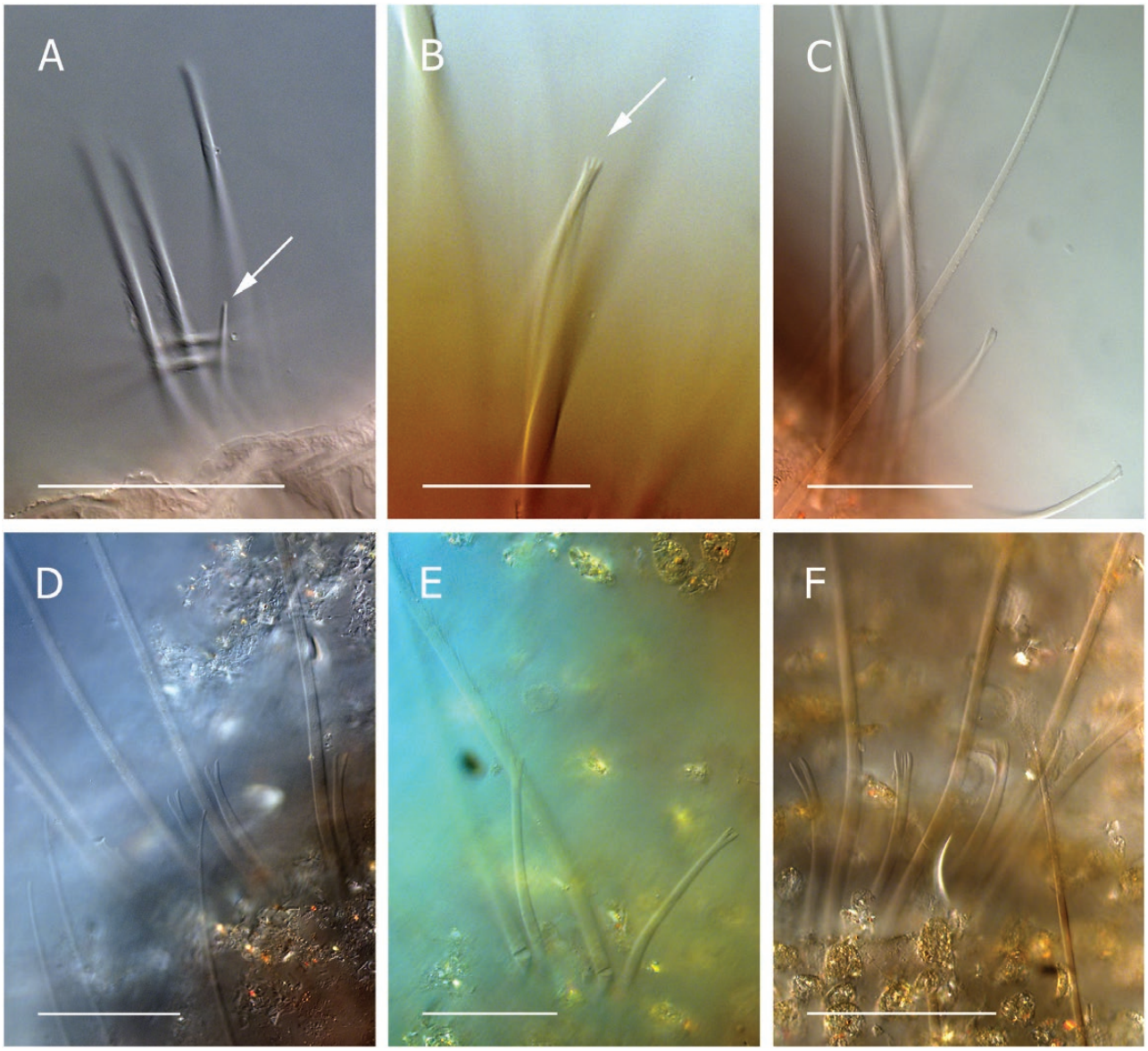


Figure 7. A–F, dorsal setae: hairs, pilose (A, C–F), smooth (B); needles, simple-pointed (A), trifid (B), pectinate (C–F). A, G4, 01.036.06. B, G5, 01.036.09. C, G6, 00.346.03. D, G6, 01.076.04. E, G7, 01.076.03. F, G7, 01.076.07. Group (G) and specimen codes refer to Supporting Information, Table S1. [Scale: A and D (100 μm); B (25 μm); C and E (200 μm).]

(testes and ovaries in early stage present), stained in paracarmine and mounted in Canada balsam. *COI* barcode sequence, GenBank acc. no. MK984610; 16S sequence, GenBank acc. no. MN000035; ITS sequence, GenBank acc. no. MK999995; H3 sequence, GenBank acc. no. MK984655.

Figure 6. A–B, crystals. A, G5, 01.065.02, one crystal in the atrium on the left side of the specimen, probably shaped in the amorphous mass surrounding it. B, same specimen as A, right side of the specimen, showing a large crystal, accompanied by a small crystal, both immersed in an amorphous mass that surrounds them. C–E, prostomia, well retracted into the mouth cavity (C, E) or slightly protruding out of the mouth cavity (D). C, *Baikalodrilus trituberculum*, G1, 00.346.01. D, G2, 01.036.03. E, G7, 00.161.01. F, *Baikalodrilus trituberculum*, left lateral side of first 17 anterior segments, showing the dorsal, dorsolateral and ventral glandular tubercles, G1, 00.346.01. Group (G) and specimen codes refer to Supporting Information, Table S1. [Scale: A–F (50 μm).]

Type locality: RUSSIA. Cape Kurly, Lake Baikal, 50 m, station 95.17, 55.6667° N, 109.4247° E (datum WGS84), sample No 336.

Other material: RBINS 01.076.06, two fragments of one immature individual: one (first 13 anterior segments) stained in paracarmine and mounted in Canada balsam, the second one (last 18 posterior segments) preserved in absolute ethanol. Akademichesky ridge, Lake Baikal, 315 m deep, station 96.57, 53.5283° N, 107.9142° E, sample B96.57. *COI* barcode sequence, GenBank acc. no. MK984611; 16S sequence, GenBank acc. no. MN000036; ITS sequence, GenBank acc. no. MK999996; H3 sequence, GenBank acc. no. MK984656.

Etymology: Noun in apposition, From Latin *tria*, three, and *tuberculum*, a small lump or swelling, in reference to the three pairs of glandular tubercles per segment, characteristics of the species.

Diagnosis: This species is unique in having three pairs of buttonhole-like glandular tubercles on the same line, one ventral, besides and in front of setae; dorsal setae midway between dorso-lateral and dorsal tubercles. It can be also distinguished from other species in *Baikalodrilus* by genetic data (Fig. 3).

Description: Length of (fixed) holotype 4.5 mm, 17 segments (anterior fragment). Maximum width 1.49 mm (dissected specimen flattened between slide and coverslip). Prostomium triangular, 120 µm long, roughly as long as wide, well retracted into the mouth cavity and located between transversal setal lines III and IV when retracted (Fig. 6C). Dorsal bundles with 1–2 smooth hair setae with straight tip, 1–2 simple pointed needles; 1–2 simple pointed setae in ventral bundles. Setae hardly visible on their full length. Ventral setae 147 µm long and 4.6 µm wide in II, 273 µm long (minimal value) and 6.4 µm wide in XV. Dorsal setae not visible on their full length. 'Hülsenpapillen' present all over the body fragments studied, sustained by small, internal, epidermal prickles. Two rows of 'Sinnespapillen' per segment (Fig. 1D). Three pairs of buttonhole-like glandular tubercles on the same line, one ventral, besides and in front of setae; dorsal setae midway between dorsolateral and dorsal tubercles (Figs 2A, 5F). Glandular tubercles weakly developed on immature individual.

Remarks: The combination of smooth hair setae, simple pointed needles and only simple pointed ventral setae is only seen in *B. discolor acinacifer* and *B. discolor discolor*. In our material, such a combination is also found in specimens of group 3 (see above). However, our specimens are genetically separated from the latter group (Fig. 3). In addition, the presence of three pairs of glandular tubercles per segment is a feature unknown in any *Baikalodrilus* species described to date.

Geographical distribution and habitat: Lake Baikal: Akademichesky ridge, cape Kurly, 50 to 315 m deep, soft sediment.

DISCUSSION

Molecular and morphological species delimitation

Our molecular methods of species delimitation are based on three different approaches: distances (ABGD), allele sharing (haploweb) and phylogenetic inference (GMYC). However, the performance of each method is variable and subject to its own errors, resulting in either oversplitting or overlumping (Dellicour & Flot, 2018). This probably explains why the different methods applied here produce different results in terms of species delineation. For instance, species delimitation based on the GMYC analysis suggests 21 candidate species, many of which that were undistinguishable by morphology. However, this method may oversplit the data (Pentinsaari *et al.*, 2017; Luo *et al.*, 2018). Given the complexity of *Baikalodrilus*, it is important to approach species delineation in this species flock with particular caution. Following the recommendations of Dellicour & Flot (2018) and Carstens *et al.* (2013), we focus our discussion of potential species on those for which the three approaches used are congruent. In this regard, it is reassuring to note that the methods did not produce conflicting results.

Molecular methods of species delimitation and morphology are congruent when ten candidate species are suggested, although this number corresponds to a level of ABGD partitioning where morphological groups 5 and 6 are not considered distinct by the latter method. In addition, ABGD, GMYC and haplowebs are congruent to suggest specimen 01.076.05 as a second candidate species in morphological group 2. These two points are discussed below.

Group 2

In group 2, the morphology of specimens 01.312.06 and 01.076.05 differs slightly from that of the six other individuals in the group. While the presence or absence of pilosity on hairs appears consistent in each of the ten groups, specimen 01.312.06 disrupts this pattern. Although similar to other individuals of group 2 in terms of hair pilosity (except specimen 01.312.06),

specimen 01.076.05 has needles with short to almost inexistent ectal teeth.

The minimum pairwise p-distance (*COI*) observed between two distinct morphospecies in *Baikalodrilus* is 5.93% [*B. digitatus* (group 8) and *B. werestschagini* (group 9)] (Table 3). Yet, the minimum p-distance between specimen 01.076.05 and any other specimen is 6.69%. This tentatively suggests a species level differentiation, even if p-distances on their own should not be interpreted as decisive taxonomic evidence, particularly not when there is no clear barcoding gap (Fig. 4). Hence, until compelling evidence indicates otherwise, we stick to the ABDG, GMYC and haploweb results, and treat specimen 01.076.05 as a new candidate species. However, we postpone its taxonomic description since only a single specimen is available, which does not allow us to properly assess intraspecific morphological variation. Specimen 01.076.05 suggests that unarmoured *Baikalodrilus* might be a complex of species differing by subtle morphological features, as implied by Snimschikova & Timm (1992) when they described *B. scaphoideus* and *B. undatus*, two large-sized species that lack papillae (see also below).

There is less support for the separation of specimen 01.312.06 from group 2 (excluding 01.076.05) as its maximal p-distance (*COI*) with the group is 3.80%. This implies that the presence/absence of pilosity on hairs is a character that can show intraspecific variability.

Group 5 and Group 6

Although ABGD did not distinguish the two groups as candidate species when the fourth level of partitioning is considered (ten candidate species), they differ from each other by three morphological characters: smooth vs. pilose hairs, simple pointed to trifid ectal tip of needles vs. pectinate tips, and only simple pointed vs. simple pointed and bifid ventral setae. Because of these morphological differences, it seems reasonable to consider the two groups as distinct species, even if they show a low minimum p-distance (4.40%). Interpreting both groups as a single species would invalidate several morphological characters that are useful for defining the other groups as candidate species. Groups 5 and 6 are identified as distinct species in the GMYC and haploweb analyses, and when higher partitions are considered in the ABGD analysis, but uncertainty is then increased as well, by involving more paraphyletic species or species that correspond to unsupported clades (Fig. 3).

Finally, ITS haplotypes were shared between morphological groups 6 and 7.2, and between 7.1 and 7.2 (Fig. 5). These connections between closely related evolutionary lineages have not been observed using the *COI* data and may suggest incomplete lineage sorting.

TAXONOMIC VALUE OF MORPHOLOGICAL CHARACTERS OF *BAIKALODRILUS*

Snimschikova & Timm (1992) provided an identification key for the 20 *Baikalodrilus* species (and two subspecies) known at that date, later updated by Semernoy (2004). These keys mainly relied on characters related to body size, setae, papillae, glandular tubercles, 'crystals' and male genitalia. As an identification key is supposed to make use of the most discriminant characters among species, these characters need to be reconsidered in the light of present results.

Somatic setae: Somatic setae are commonly used as a source of specific criteria, not only for *Baikalodrilus* species (Snimschikova & Timm, 1992), but also for the former '*Peloscolex*' species complex and for oligochaetes in general (Brinkhurst & Jamieson, 1971). However, it is well known that oligochaete somatic setae may strongly depend on abiotic conditions (pH, salinity, water hardness; Loden & Harman, 1980; Chapman & Brinkhurst, 1987), which raises the question of how relevant setal characters are with regard to *Baikalodrilus* taxonomy.

Pilosity of hair setae: The presence or absence of pilosity on hair setae seems to be one of the most constant taxonomic characters within the different groups of *Baikalodrilus*. One specimen (01.312.06) in group 2 seems to deviate from this rule, but in that case, the possibility that a second distinct taxon is involved cannot be discarded.

Number of setae: This feature seems to at best provide partial discrimination, because some groups have few setae per bundle (1–2), while others display up to seven setae. Group 7 is characterized by the maximum number of hair setae and ventral setae reached in anteclitellar bundles, which constitutes a unique feature for the group. Therefore, even if the number of setae seems to provide valid taxonomic information, it should always be used in combination with other characters.

Ectal tip of needles: Either only simple pointed or only bifid needles, is a constant feature for each group, except for group 5, where slightly bifid to simple pointed needles can be seen on the same individual, as well as simple pointed needles with small ectal notches giving a 'trifid' appearance to the needle (Fig. 7B). As such this feature is discriminating (Table 4), although it does not constitute a characteristic of any group except for group 5 where individuals possess both types of needles.

Types of ventral setae in the same bundle: In *Baikalodrilus* species, ventral setae can be of two types, bifid or simple pointed, one type being exclusively present in all bundles or both co-occurring in anteclitellar bundles (Snimschikova & Timm, 1992). As a rule, setae are always simple pointed when only one type is present. *Baikalodrilus inflatus* is the sole species mentioned with only bifid setae in all ventral bundles. However, it is likely that Michaelsen (1901) did not first notice simple pointed setae as, four years later, their presence was added to the species diagnosis, although they were 'exceptionally simple pointed' ('ausnahmsweise einfach-spitzig') (Michaelsen, 1905: 23). Brinkhurst (1981b) was unable to clarify this issue from his re-examination of Michaelsen's type material from the Zoological Museum of the University of Hamburg (ZMUH), since setae in the specimens examined were mostly broken. Hrabě (1982) gave an emended description of the species, from new, non-type material, in which he distinguished two forms of setae in anteclitellar bundles. In his examination of additional specimens from ZMUH, Brinkhurst (1984: 499) noticed ventral setae both simple pointed and bifid. Hence, it seems that the only exception to 'setae always simple pointed, when only one type is present' is due to an inaccurate original observation, which would invalidate the exception. However, Snimschikova & Timm (1992: 74) questioned emended descriptions of *B. inflatus* by Hrabě (1982) and Brinkhurst (1984), suggesting that they dealt '…with different large species of the genus *Baikalodrilus* or even with a mixture of species'.

The new *Baikalodrilus* material studied here provides an interesting perspective for assessing the relevance of this character. All specimens of group 7 have both simple pointed and bifid setae in anteclitellar bundles, except specimen 01.076.07 in which all ventral setae are distinctly bifid. Similarly, all specimens of group 5 have two types of setae in anteclitellar ventral bundles, except specimen 01.036.09 in which all setae are simple-pointed. Since we cannot exclude variability for this characteristic in some candidate species, it is clear that the types of ventral setae in the same cluster are not totally reliable for distinguishing species.

Papillae and cutaneous cover: Snimschikova & Timm (1992) consider variation in papillation as interspecific rather than intraspecific. However, seasonal intraspecific variation is known to occur in armoured tubificids, such as *Spirosperma ferox* Eisen, 1879, where the 'papillae' can be shed periodically, or in relation to maturity in *Tubificoides benedii* (d'Udekem, 1855) (Dahl, 1960), which led Brinkhurst (1964, 1966b, 1981b) to invalidate the nature of the body wall as a specific criterion. In her revision of the *Peloscolex* complex, Holmquist (1978: 197) noted that the cutaneous cover was never found lacking in any coated specimens she studied, although it may possibly become thin, or is absent on newly hatched specimens. Our observations show that the absence of a cutaneous cover might be a specific character, as suggested by its constant absence in all specimens of group 2, although we cannot exclude a periodical, infrequent, shedding of this cover in other *Baikalodrilus* species, as suggested from one specimen of group 6. Hence, we cannot be so definite as Brinkhurst (1964, 1966b, 1981b) in rejecting this character as a specific criterion. Yet, it should only be used cautiously in combination with other morphological features.

Glandular tubercles: Originally referred to as setal tubercles ('Borstentuberkeln') (Michaelsen, 1901: 143), such structures appear on all specimens here studied as oval, opaline thickening of the cutaneous cover, made of large, vacuolized cells, in the close vicinity of setal bundles, on the longitudinal setal line (Fig. 2), with their longer axis crosswise to the body axis. There are four tubercles per segment, but sometimes duplicated and surrounding setal bundles (Fig. 2E). In group 1, there are three pairs of glandular tubercles, one ventral, close to setal bundles, one laterodorsal and one dorsal, with dorsal setal bundles in between. In addition, they are clearly distinct from setal glands, when the latter are present (Fig. 2F), hence referring to them as 'setal tubercles' (e.g. Michaelsen, 1901; Snimschikova & Timm, 1992) is misleading. The size and distinctness of glandular tubercles are variable features, the evaluation of which is often hindered by the thickness of the cutaneous cover and difficult to objectivize. In addition, intragroup variation is also possible (see group 1), which makes this character of low taxonomic value.

These tubercles are assumed to have a glandular function (Chekanovskaya, 1975; Hrabě, 1982). In *B. werestschagini*, Michaelsen (1933, 1935) regarded them as sensory and coined the term 'Sinneshügel' to designate them. In this respect, it is interesting to note that glandular tubercles are present and well developed in all specimens of morphological group 2, whereas 'Hülsenpapillen' are absent in the group. So, if the 'glandular' tubercles have a glandular function indeed, it does not contribute to the formation of 'Hülsenpapillen'. On the other hand, the almost constant close association of glandular tubercles with setae (except for dorsal glandular tubercles in *B. trituberculum*) suggests that their role must be interpreted taking this observation into account. As suggested by Michaelsen (1933, 1935), these structures may have a sensory function but their role remains purely speculative.

'Crystals': The presence of shuttle-shaped, crystal-like bodies in the atrium is unique to the genus *Baikalodrilus*, which led Snimschikova (1984) to establish the subgenus *Crystallifer* ('crystal bearing') (now considered as junior synonym of *Baikalodrilus*), referring to this distinctive character. Timm (1998) considered this feature as a distinct synapomorphy of the genus. However, the term 'crystal' is misleading since both its structure and composition are unknown. This atrial body was first mentioned as a 'Gallertstab' by Michaelsen (1933, 1935) in the description of *Peloscolex werestschagini*, possibly by analogy with the so-called crystalline style in bivalves and gastropods. 'Gallertstab', or 'gelatinous rod', is indeed a term used in the old German biological literature to designate such a structure (von Fürth, 1903), the word 'Kristallstiel' being preferred nowadays (Götting, 1996). In molluscs, it involves a rod-like matrix of proteins that aids in digestion (Kristensen, 1972).

In *Baikalodrilus*, an organic origin of the 'crystals' is also suspected, probably from the prostatic glands (Snimschikova & Timm, 1992). In specimen 01.065.02 (group 5), there is one large crystal in each atrium, immersed in an amorphous, granular mass containing a multitude of small crystals (Fig. 6A, B). In one atrium, one crystal is half the size of the large one (Fig. 6B). This observation is interesting as it gives clues on the possible origin of crystals, their shaping and their taxonomical value for species discrimination. The granularity suggests that such an amorphous mass is indeed of prostatic origin. This material would then be rearranged in crystal-like bodies, shaped by the atrium, and progressively incorporated into a large final crystal, without any remains of the original amorphous mass. It is probably not a coincidence that crystals fit the shape of the atrium, as early noted by Michaelsen (1933: 332) [italics are ours]: 'Das Lumen dieses dickeren Atriumteiles ist ziemlich eng und enthält (bei den Organen beider Seiten) einen eigentümlichen, in Hämatoxylin-Eosin tief rot gefärbten dünnen, ca. 5 µ dicken, glatten, *der Krümmung des Atriums entsprechend gekrümmten Stab*, der wie ein Gallertstab aussieht.' ['The lumen of this thicker part of the atrium is rather narrow and contains (in the organs of both sides), a peculiar thin, smooth rod, about 5 µm thick, coloured deep red in haematoxylin-eosin, and *curved like the curvature of the atrium*, which looks like a gelatine rod.'] Hence, the frequent use of the number of crystals by Snimschikova & Timm (1992) is questionable as a distinctive character for a species. In any case, the absence of a large, secondary crystal in one atrium of the same species, while present in the other atrium, shows the intraspecific variability of this character.

The location of a group of crystals outside the atrium is a distinctive feature of *B. solitarius* (Snimschikova, 1982: 98), although Snimschikova & Timm (1992) admitted that such a location was problematic and might be an artefact. It is indeed difficult to believe in crystals in the cœlom and not the atrium, as in all other *Baikalodrilus* species known to date, all the more so since this observation was based on only one fragment consisting of 14 anterior segments. In 1993, one of us (PM) saw Snimschikova's *Baikalodrilus* type material and noted the bad state of conservation of the *B. solitarius* tissue fragment, mounted in glycerine, and suggested that the location of crystals in the coelomic cavity was indeed an artefact.

Male genitalia: Snimschikova & Timm (1992) frequently make use of characters related to male genitalia as distinctive characters for species, while admitting that 'all species exhibit a similar reproductive system'. Usually, some of these characters can provide valuable taxonomic information in Oligochaeta, e.g. characters related to vasa deferentia (length, thickness), atria (size, shape, width, thickness), spermathecal ducts (length) and prostates (location of opening on atria). In practice, the male ducts of *Baikalodrilus* species are similar among species, a similarity noted in early studies of this genus (Michaelsen, 1933, 1935; Brinkhurst, 1981a, b, 1984). In one of the last taxonomic works published on the genus *Baikalodrilus*, Timm (1998: 31) wrote that 'internal reproductive organs are quite uniformous [sic]', implicitly admitting that sexual characters have little taxonomic value in the genus. Other genital characters are clearly questionable, such as the number, shape and location of 'crystals', or the shape of spermathecal ampullae, the latter being usually dependent on their degree of filling (Holmquist, 1978).

In summary: There are few morphological characters that can be used as distinctive, specific criteria in the genus *Baikalodrilus*. The pilosity of hairs is probably one of them, if some credibility is given to the assumption that two individuals of group 2 are actually different species (see above). The discriminant nature of other characters is variable, they need to be used in combination with others (see Table 4).

Association between groups and nominal species

A reassessment of *Baikalodrilus* species is needed, in particular for the taxa described by Snimschikova (1982, 1984, 1989a, 1989b, 1991b) (Table 1). It is striking that the description of these large species does not fit most of our *Baikalodrilus* specimens. Among these ten species, a few have distinctive features, which leaves little doubt about their validity as a species with the current knowledge of the genus.

Both *B. bekmanae* and *B. falcatus* have characteristic sickle-shaped setae in ventral bundles (sigmoid in other species), only present in posterior segments and absent in midbody (IX–XIX) in *B. bekmanae,* while present in all segments in *B. falcatus*.

Other species are more questionable. *Baikalodrilus solitarius* is remarkable in having clusters of crystals outside the atrium, but this feature is probably an artefact. Other species differ by subtle and overlapping features related to the body size, number of segments or setae per bundle. The assessment of intraspecific variability is often hampered by the fact that descriptions of many species are based on few or single specimens, some of which are not mature or are available as a fragment only. A tentative assignment of the different *Baikalodrilus* groups, identified in this study, to known species could help to clarify some issues and to take taxonomic decisions as to some of these species, as seen below.

Group 2 – Baikalodrilus *cf.* paradoxus

The constant absence of 'Hülsenpapillen' is known in eight *Baikalodrilus* species so far, five small-sized species (*B. exilis*, *B. intermedius*, *B. parilis*, *B. phreodriloides* and *B. vicinus*) and three large-sized species (*B. paradoxus*, *B. scaphoideus* and *B. undatus*). Considering only the latter group, *B. paradoxus* has dense papillae fused into a regular layer in contrast to the other two species, which have no papillae at all. In the original description, the cutaneous cover of *B. paradoxus* looks similar to what can be seen on specimens from group 2 (Snimschikova, 1984: 10; Fig. 3G). The so-called papillae in *B. paradoxus* probably correspond to fine transverse ringlets of the cutaneous cover.

Baikalodrilus paradoxus, *B. scaphoideus* and *B. undatus* are similar species that differ in adult body length (20–24 mm, 14–16 mm and min. 6 mm – single fragment, respectively), the number of ventral setae per bundle in anteclitellar segments (3–4 vs. 2 and 2, respectively), the number of crystals in each atrium (1 in *B. paradoxus* and *B. scaphoideus* and 2 in *B. undatus*) and the presence of small, poorly developed vs. large glandular tubercles in *B. scaphoideus* or *B. undatus* vs. *B. paradoxus.* However, these features, or their range of variability, are either subtle or questionable as criteria for species identification. The validity of *B. scaphoideus* and *B. undatus* as distinct species from *B. paradoxus* remains questionable and it seems better to consider them as junior synonyms of *B. paradoxus*. Group 2 is consistent with *B. paradoxus*, if we admit that pectinate needles described in the latter species correspond actually to bifid needles with duplicated upper tooth, as seen on our material.

Group 3 – Baikalodrilus discolor *and its two subspecies*

The combination of smooth hair setae, simple pointed needles, only simple pointed ventral setae and two pairs of glandular tubercles per segment is typical of *B. discolor acinacifer* and *B. discolor discolor*. Regardless of the meaning of subspecific taxa in a lake where other sympatric taxa of *Baikalodrilus* are regarded as separate species, the two subspecies differ only in the number of crystals in the atrium (2 and 1, respectively). This feature is deemed unreliable, hence we consider *B. discolor acinacifer* as a junior synonym of *B. discolor discolor*.

Group 4 – Baikalodrilus malevici

Pilose hair setae, simple pointed needles, only simple pointed ventral setae, and 'Hülsenpapillen' present are features only seen in *B. malevici*, *B. multicrystallifer* and *B. solitarius*. These three species differ in body size, number of setae per bundle, shape of spermathecae and atria, and number and location of crystals (Snimschikova & Timm, 1992). Such differences often rely on overlapping values (body size, number of needles and ventral setae), are subtle and probably depend on sexual maturity (shape of spermathecal ampullae, atria, number of crystals in atria), result from an artefact (crystals outside atrial lumen) or are based on a single, fragmentary specimen, which prevents the evaluation of intraspecific variability (*B. solitarius*). Consequently, it seems preferable to consider *B. multicrystallifer* and *B. solitarius* as junior synonyms of *B. malevici*. Group 4 can be ascribed to *B. malevici* although there are fewer hairs per anteclitellar segment in specimens of group 4 than in *B. malevici* (1–4 vs. 5–6).

Group 5 – Baikalodrilus sp.

Body size longer than 4 mm, smooth hairs, absence of pectination on needles, sigmoid ventral setae, and only simple pointed ventral setae are only known in *B. discolor*. However, the ectal tip of needles is variable in group 5, from bluntly simple pointed to bifid with short teeth 4 µm long, and sometimes with simple pointed with ectal small notches. *Baikalodrilus medianus* displays a similar variability in ectal shape of needles. This species has also both simple pointed and bifid setae in ventral bundles, as specimens 01.065.02 and 01.065.03, in contrast to the other specimen 01.036.09 constitutive of Group 5 (only simple pointed setae). However, *B. medianus* has pilose hairs and the presence or absence of pilosity on hair setae is one of the most constant specific characters within the different groups of *Baikalodrilus*. Specimens of group

5 share with *B. multicrystallifer* and *B. solitarius* small secondary crystals but the latter two species have slightly pilose hairs as well. Hence, the assignment to extant species remains an unsettled challenge as the type material cannot be re-examined.

Groups 6 and 7 – Baikalodrilus inflatus *species complex*

Both groups have, basically, a similar morphology, although they can be clearly distinguished in the phylogenetic tree (Fig. 3), with high support values. All specimens have pilose hairs, ventral setae of two types, 'Hülsenpapillen' and pectinate needles, which fit the description of *B. inflatus* (whether emended by Brinkhurst (1984) or Hrabě (1982)), *B. crassus* and *B. medianus*. However, these three species are essentially indistinguishable according to their description.

Baikalodrilus crassus is said to be most closely related to *B. inflatus*, the latter differing by a longer and half as wide body, only bifid crotchets in ventral bundles and a smaller number of setae of all types (Snimschikova & Timm, 1992). According to our revision of the taxonomic value of morphological characters (see discussion above, among others 'Somatic setae'), such differences are too subtle to have a specific value. In contrast, besides morphological peculiarities linked to the body size, 'Hülsenpapillen', spermathecae and atria, *B. medianus* has two types of needles, either pectinate or bifid, the latter with some intermediate teeth in anteclitellar bundles, a morphological peculiarity not visible in specimens of groups 6 and 7, although documented in *B. inflatus sensu* Hrabě, 1982.

While groups 6 and 7 fit the description of *B. inflatus*, they are probably different species. This observation gives weight to Snimschikova & Timm (1992) and Semernoy (2004: 271–273) when they assumed that *B. inflatus* was actually described from a complex of different species. Type material of *B. inflatus* is still present in the Hamburg Museum, although its state of conservation does not enable us to solve the issue (e.g. mostly broken setae in all bundles). As a result, we agree with Snimschikova & Timm (1992) in considering this taxon as a *species inquirenda*. To stabilize nomenclature, a neotype should be designated, preferably from a station in the vicinity of the type locality and which includes DNA typification.

Given their morphological resemblance, it would be logical to consider *B. crassus* and *B. medianus* as junior synonyms of *B. inflatus*. But as the latter species is itself poorly defined, the problem remains unresolved pending a neotypification of the species. In the meantime, we have no other choice but to consider *B. crassus* and *B. medianus* as *species inquirendae* as well.

Taxonomic implications

From the re-assessment of the taxonomic value of morphological characters of *Baikalodrilus*, and a tentative association between groups and nominal species, we conclude that, for at least the large-bodied species of *Baikalodrilus*, an identification based on morphology will often be reduced to a search for small details, whose diagnostic value remains difficult to evaluate. Difficulty in separating species by morphology may be related to the young age of a species flock, so that it is difficult to distinguish separately or only partially evolving lineages [according to the unified species concept of de Queiroz (2007)]. The validity of many *Baikalodrilus* species remain questionable. Unfortunately, Snimschikova's type material was not deposited in the Limnological Institute of Irkutsk (Russia). Currently, it remains untraceable and the possibility is high that it is lost.

Increasing the *Baikalodrilus* dataset by sampling more stations at different locations and at different depths of the lake remains highly desirable, in order to hope for a clearer understanding of this species flock. Until this is done, it is to be feared that the systematics of *Baikalodrilus* will be reduced to a few species with an easily characterized morphology, plus a group of indistinguishable species, for which we have no other choice but to refer to as *Baikalodrilus* spp.

Meanwhile, we here summarize the systematics of *Baikalodrilus*, incorporating the different taxonomic decisions resulting from the present study.

Systematics

Family Naididae Ehrenberg, 1828

Subfamily Tubificinae Eisen, 1879

Genus *Baikalodrilus* Holmquist, 1978

Type species: Peloscolex kozovi Hrabě, 1969 = *Baikalodrilus kozovi* (Hrabě, 1969).

Synonym: Crystallifer Snimschikova, 1984: 15.

Baikalodrilus alienus Timm, 1998: 27–31, figs 16–44.

Baikalodrilus bekmanae (Snimschikova, 1984). Protonym: *Peloscolex bekmani* Snimschikova, 1984: 6–9; fig. 2.

Baikalodrilus bifidus Snimschikova, 1989b: 26–28; fig. 3.

Protonym: *Baikalodrilus discolor brevipectinatus* Snimschikova, 1989b: 32–33; fig. 6.

Baikalodrilus cristatus (Snimschikova, 1982). Protonym: *Peloscolex cristatus* Snimschikova, 1982: 95–96; fig. 6.

Baikalodrilus discolor (Snimschikova, 1984). Protonym: *Peloscolex discolor* Snimschikova, 1984: 3–6; fig. 1.

Synonym: Baikalodrilus discolor acinacifer Snimschikova, 1989b: 30–32; fig. 5.

Baikalodrilus digitatus Holmquist, 1979: 50–51; figs 16B–D, 17.

Baikalodrilus dividus Semernoy, 2004: 314–315; fig. 175.

Baikalodrilus exilis (Snimschikova, 1982). Protonym: *Peloscolex exilis* Snimschikova, 1982: 93–95; fig. 5.

Baikalodrilus falcatus (Snimschikova, 1982). Protonym: *Peloscolex falcatus* Snimschikova, 1982: 98–99; fig. 8.

Baikalodrilus intermedius Snimschikova, 1991b: 134–136; fig. 1.

Baikalodrilus kozovi (Hrabě, 1969). *Basionym: Peloscolex kozovi* Hrabě, 1969: 269–272; figs 1–6.

Baikalodrilus malevici (Chekanovskaya, 1975). Protonym: *Peloscolex malevici* Chekanovskaya, 1975: 128–130; fig. 7.

Synonyms: Baikalodrilus multicrystallifer Snimschikova, 1989a: 300–302; fig. 1. *Peloscolex solitarius* Snimschikova, 1982: 96–98; fig. 7.

Baikalodrilus paradoxus (Snimschikova, 1984). Protonym: *Peloscolex paradoxus* Snimschikova, 1984: 9–12; fig. 3.

Synonym: Baikalodrilus scaphoideus Snimschikova, 1989b: 23–25; fig. 1.

Synonym: Baikalodrilus undatus Snimschikova, 1989b: 25–26; fig. 2.

Baikalodrilus parilis Semernoy, 2004: 287–289; fig. 155.

Baikalodrilus phreodriloides (Michaelsen, 1905). Protonym: *Lycodrilus phreodriloides* Michaelsen, 1905: 16–18.

Baikalodrilus scaphoideus Snimschikova, 1989b: 23–25; fig. 1.

Baikalodrilus solitarius (Snimschikova, 1982). Protonym: *Peloscolex solitarius* Snimschikova, 1982: 96–98; fig. 7.

Baikalodrilus trituberculum Martin (present study).

Baikalodrilus undatus Snimschikova, 1989b: 25–26; fig. 2.

Baikalodrilus vicinus Semernoy, 2004: 315–318; fig. 174.

Baikalodrilus werestschagini (Michaelsen, 1933). Protonym: *Peloscolex werestschagini* Michaelsen, 1933: 327–333; figs 1–3.

Species and subspecies inquirendae

Baikalodrilus crassus Snimschikova, 1989b: 28–29; fig. 4.

Baikalodrilus medianus Snimschikova, 1991b: 136–137; fig. 1.

Baikalodrilus inflatus (Michaelsen, 1901). *Basionym: Tubifex inflatus* Michaelsen, 1901: 141–145; figs 8–10.

Remark: Probably a species complex.

Remarks: Baikalodrilus discolor brevipectinatus is likely a species on its own, distinct from *B. discolor s.s.* by hairs (pilose vs. smooth) and ectal tip of needles (pectinate vs. simple pointed). These morphological characters were shown to be significant at the specific level.

EVOLUTIONARY IMPLICATIONS

Phylogenetic analyses provide valuable insights into evolutionary patterns of the species flock. Two aspects can be addressed: the timing of the evolutionary radiation of the group in the geological history of Lake Baikal, and evolution of body size in the evolutionary history of the species flock.

Divergence time between species

The time-calibrated *COI* tree suggested that the most basal split in *Baikalodrilus* occurred between 2.5 and 5.3 Mya and that most lineages corresponding to species diverged between 0.9 and1.8 Mya. Although these estimates must be made with all due caution, they suggest that the species flock is of recent origin, much younger than the lake itself. Even tough ancient radiations can still be distinguished in Lake Baikal, it is usually acknowledged that a general cooling at the beginning of the Pleistocene caused major environmental changes, which caused a general rearrangement of the Baikal fauna (Sherbakov, 1999). Hence, it is remarkable that divergence time estimates within the *Baikalodrilus* species flock are of the same order of magnitude as those of other young Baikal flocks. It has been estimated to 2 Mya for Baicaliidae gastropods (Zubakov *et al.*, 1997), between 3 Mya (Kiril'chik & Slobodyanyuk, 1997) to 6.5 Mya (Kontula *et al.*, 2003) for Baikal cottoids, around 5.3 Mya for the majority of Baikal *Cytherissa*

ostracods (Schön & Martens, 2011) and to 2.3 Mya for the Baikal endemic sponge family Lubomirskiidae (Maikova *et al.*, 2015).

Snimschikova & Timm (1992) regarded the Palaearctic genus *Embolocephalus* Randolph, 1892 as the closest relative to *Baikalodrilus* and assumed that both genera evolved from a common ancestor. In our phylogeny, *Embolocephalus* species are also the closest sister-group to *Baikalodrilus* among the selected outgroup taxa. *Embolocephalus* species are widespread in the Palaearctic region and are usually found in cold, well-oxygenated and oligotrophic freshwater, some of them being found in the profundal of lakes or in water bodies that have links with groundwater (Lafont *et al.*, 2006; Timm, 2009; Ohtaka & Martin, 2011; Van Haaren & Soors, 2013). Therefore, we assume that the general cooling that occurred at the beginning of the Pleistocene created favourable environmental conditions for the ancestral form of *Baikalodrilus* to live in Lake Baikal and radiate to form the species flock that currently exists.

Body size

In their key to *Baikalodrilus* species, Snimschikova & Timm (1992) split species into two groups of body size, with a threshold around 4 mm, suggesting a size gap within the species flock. Species as small as 4 mm or less are unknown in other papillate genera related to *Baikalodrilus* (i.e. *Embolocephalus*, *Quistadrilus* and *Spirosperma*), implying that this feature is an apomorphy. Ranking the body size range for each species according to increasing values suggests indeed a small gap around 5 mm, although there is no clear-cut threshold between small-sized species and large ones (Fig. 8). The three small species included in this study do not only group together in the phylogenetic tree, but also are sister to all other *Baikalodrilus*, suggesting that such a split occurred early in the course of evolution of the flock. Snimschikova & Timm (1992) suggested that small size facilitated sheltering in coarse littoral sediment and that the body size increased with increasing bathymetric depth. Since then, at least two different small *Baikalodrilus* species, *B. digitatus* and *B. cristatus*, were found not

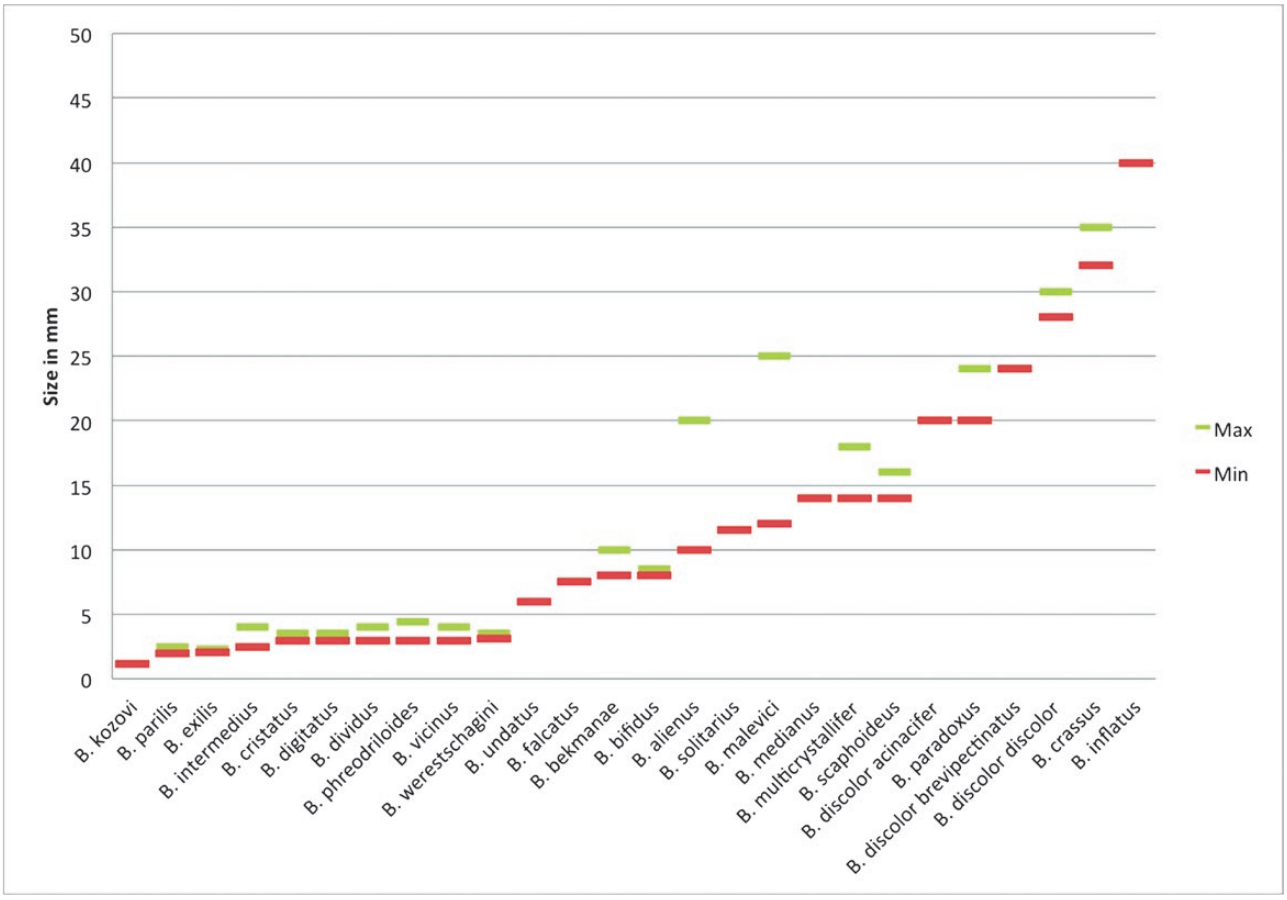


Figure 8. Distribution of body size range of *Baikalodrilus* species (in taxonomic acceptance prior to this study) according to increasing minimal values (see Table 1).

only in soft sediment, but also in the abyssal zone of Lake Baikal [namely below the upper 250 m, which corresponds to the dimictic zone; *B. digitatus* having even been reported from 1250 m deep; Martin *et al.* (1999)], which give no support to such an evolutionary scenario. Further molecular studies, including better taxonomic representativeness of the flock, in particular small species, are needed to confirm the existence of a size threshold within the species flock.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Location of the stations where the different specimens of *Baikalodrilus* were found. Numbers refer to specimens as listed in Supporting Information, Table S1.

Table S1. List of specimens included in the study, general inventory numbers (I.G.), voucher numbers (N/V: no voucher), DNA identifier number, sampling data (name of locality, country, depth of sampling, station code, latitude, longitude – datum WGS84, sampling code, collector) and dates, GenBank accession Nos. Abbreviations: PM, Patrick Martin; AO, Akifumi Ohtaka.

Table S2. Support values for nodes of phylogenetic trees obtained using a combination of three different datasets (two concatenated sets: *COI*_16S_ITS, *COI*_16S; ITS), three phylogenetic inference methods (Bayesian inference – BI, maximum likelihood – ML, and maximum parsimony – MP), and three different alignment methods (ITS only: LocARNA, Q-INS-I, ClustalX; default alignment method for other gene fragments: ClustalX). Node labels refer to labels illustrated in Figure 3. The symbol '<' means that posterior probabilities (BI) or bootstrap values (ML, MP) are below 0.95 or 70, respectively (thresholds for support values according to San Mauro & Agorreta, 2010).