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Early Development and Post Embryonic Skeletal Morphology of the Progeny of Spined Loach *Cobitis taenia* L. (Teleostei, Cobitidae) and its Naturally Occurring Allotriploids*

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Polyploid fishes of the genus *Cobitis* represent a valuable model system to study the origin and consequences of hybridization and polyploidization within vertebrates. These naturally accessible polyploids are an excellent subject to determine the advantages or disadvantages of polyploidy. We investigated the embryonic and larval development with skeletal morphology of diploid and polyploid *Cobitis* progeny, obtained from crosses between females and males of *Cobitis taenia* and between allotriploid *Cobitis* females and *C. taenia* males. Observations were made during first fourteen days post fertilization. The pattern of development of all investigated individuals was the same. However the diploids developed synchronically, achieving successive stages faster than the polyploid ones; hatching was observed at 50 and 63 hours post fertilization, respectively. Statistically significant differences in hatching success and survival rate between diploid and polyploid progeny were not observed. All newly hatched larvae were characterized by a large amount of yolk, forty myomeres, body pigmentation and four external gills. Skeletal elements of the chondrocranium in the first days post hatching consisted of the otic capsule, ethmoid plate, trabeculae cranii and Meckel's cartilage. In contrast to the diploids, the polyploid larvae were characterized by a higher number of deformities. This study gives new comparative data on the features of early development of diploid and polyploid *Cobitis* progeny.

Key words: Embryonic development, deformities, skeleton, larvae, loaches, polyploids.

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Polyploid organisms with one or more additional chromosome sets are frequent among plants, occur also in invertebrates, but are rare in vertebrates, known in fish and amphibians (OTTO & WHITTON 2000; LEGGATT & IWAMA 2003). Some polyploid fishes, e. g. taxa of the genus *Cobitis* distributed in Central Europe, represent a valuable model system to investigate the origin and consequences of hybridization and polyploidization (JANKO *et al.* 2012).

Polyploid fish taxa are usually phenotypically very similar to their diploid relatives. Their tissues are built of fewer but larger cells in comparison to diploid ones, the reduced number of cells helps to maintain an almost unchanged size of organs as well as whole body (BENFEY 1999; COMAI 2005). Polyploidy induces redundant genes, increasing the possibility of gene loss, gene silencing, evolution of new genes and more possibilities for new characteristics and adaptations (OTTO & WHITTON 2000; LEGGATT & IWAMA 2003; COMAI 2005).

The effect of ploidy on growth rate, survival, skeletal deformities, reproduction, viability of eggs and developing embryos mainly of commercially important fish species have been studied (BENFEY 1999; FELIP *et al.* 2001; TIWARY *et al.* 2004; MAXIME 2008; PIFERRER *et al.* 2009; FJELLDAL & HANSEN 2010; FRASER *et al.* 2013).

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The physical or chemical manipulation of ploidy level induces a higher incidence or severity of deformities and lower survival of triploid larvae (PIFERRER *et al.* 2009). However, there is little data on the consequences of naturally occurring polyploidy which does not display decreased performance often observed in artificially produced polyploids (BENFEY 1999).

Most *Cobitis* taxa distributed in Europe are polyploids and have originated as the result of double hybridization of the spined loach *C. taenia* Linnaeus, 1758 with related species (VASIL'EV *et al.* 1989; BOROŃ 2003; JANKO *et al.* 2007, 2012; JUCHNO *et al.* 2007). Long term karyological verification of 24 *Cobitis* populations distributed throughout Poland demonstrates that 20 of them contain diploid-polyploid complexes and the remaining four populations are composed of an exclusively diploid *C. taenia* (BOROŃ 2003). Therefore, *C. taenia* appears mainly in mixed, diploid-tetraploid *Cobitis* populations dominated by triploid hybrid females (BOROŃ 2003; JANKO *et al.* 2007; JUCHNO *et al.* 2007). This species is not distinguishable by external features from its polyploid hybrid forms, so other methods e.g. karyotype consisting of 2n = 48 chromosomes are used in identification (VASIL'EV *et al.* 1989; BOROŃ 2003).

Previously conducted studies clearly revealed that some of the reproductive biology features of triploid hybrid *Cobitis* females may explain their dominance in mixed populations. They reproduce mainly gynogenetically (VASIL'EV *et al.* 1989; JANKO *et al.* 2007; JUCHNO *et al.* 2014) having a spawning time longer than *C. taenia* co-existing species and producing fewer but larger eggs that develop into larger progeny (JUCHNO *et al.* 2007, 2013). The former results and a lack of knowledge about the early ontogeny and skeletal development of taxonomically verified *C. taenia* (KOCHANOVA 1957) and its polyploid taxa lead us to the present comparative studies on the early development and skeletal morphology of diploid and polyploid progeny of *C. taenia* and allotriploid *Cobitis* females. Similar data are known for *C. melanoleuca* and its triploid clonal form (PAVLOV *et al.* 2004) and on other cobitid species distributed in Asia: *C. takatsuensis* (SHIMIZU *et al.* 1998), *Niwaella multifasciata* (KIM & LEE 1995), *Misgurnus anguillicaudatus* (ICHIYANAGI & FUJITA 1995; FUJIMOTO *et al.* 2006), *Iksookimia koreensis* (KO *et al.* 2012) and in Europe: *M. fossilis* (KRÓL *et al.* 2010). Furthermore, osteology and myology of *C. keyvani* during early ontogeny was analyzed by JALILI *et al.* (2014) and development of the hyoid and gill arches of five cobitid species were described by MABEE *et al.* (2011).

In this study we describe, for the first time, embryonic and larval development along with the skeletal morphology of experimentally obtained diploid and polyploid *Cobitis* taxa. We have used progeny of karyologically identified *C. taenia* (2n=48) and allotriploid *Cobitis* females (3n=74) from diploid-tetraploid population. The results indicate that the larger genome induces a longer period of embryonic development in polyploids and disturbances in their development expressed through more frequent deformities in comparison to diploids. The results shed light on understanding the reasons for the dominance of triploid females and the functioning of diploid-polyploid *Cobitis* populations. They also seem to be important in conservative aquaculture of declining *C. taenia* protected mainly due to the deterioration of their natural habitats (IUCN 2015).

Material and Methods

Embryonic and larval development

Females and males of the spined loach *C. taenia* were caught in June 2010 from Lake Legińskie (52°509 N), 20°559 E), whereas triploid *Cobitis* females from the diploid-polyploid population in Bug River (52°09 N), 23°309 E). The *C. taenia* individuals inhabiting the Bug River occur rarely so we used others of the same species from an exclusively diploid population in Lake Legińskie.

Experimental crosses were made by a routine fertilization method described in details by KUJAWA *et al.* (2002) and JUCHNO *et al.* (2014). We performed seven crosses between *C. taenia* females and males and six crosses between 3n *Cobitis* females and *C. taenia* males. The ploidy level of all parental individuals was identified karyologically. The chromosome slides were made according to the description by BOROŃ (1995). We used karyotype structure to identify the genome composition of triploid females. The karyotypes of *C. elongatoides* and *C. tanaitica* were adapted from MAJTÁNOVÁ *et al.* (2016), but the karyotype of *C. taenia* from VASIL'EV *et al.* 1989 and BOROŃ 2003. The matured males of *Cobitis* were identified by the presence of a *lamina circularis*, a bony plate at the base of the pectoral fin ray.

All progeny were kept in indoor aquaria well aerated at 24°C under artificial light with a photoperiod similar to natural light conditions, and fed ad libitum with *Artemia sp*. In order to describe the developmental stages, embryos and larvae were observed during the first 14 days post fertilization (dpf) and photographed using a stereomicroscope (Olympus) equipped with digital camera. The developmental stages were defined on the basis of morphological features described for *Danio rerio* by KIMMEL *et al.* (1995). Number of larvae as well as their survival rate at 14 and 21 days post hatch-

ing (dph) were calculated and compared statistically using one-way ANOVA.

Post embryonic skeletal development

The skeleton formation of 27 diploid and 71 polyploid larvae aged from 1 to 11 dph were analyzed and compared. After fixation in buffered 4% paraformaldehyde (PFA) the larvae were transferred directly to 70% ethanol, then enzymatically cleared with trypsin buffer solution (30% sodium borate + trypsin) at 20-30°C and differentially stained with alcian blue 8GX for cartilage and alizarin red S for bone (calcified structures) following the technique of DINGERKUS & UHLER (1977) with modifications by Gloria Arratia (pers. comm.).

The cleared and stained specimens were observed and photographed using a microscope Olympus CX 41 with a camera UI-1540-c and processed using Cell B, ver. 2.8 serial no. A842501-5817C380, registered to the Department of Zoology.

Fish sampling and valid animal use protocols for experiments were performed with the permissions of the Polish Ministry of Environment (no. DLOPiK-op/Ozgi-4201/V-5/ 5164/07/aj.) and the Local Ethics Committee of Poland (no. 37/2007, 20/01).

Results

The analysis of genome composition revealed that triploid females from the Bug River had karyotype (3*n* = 74; 59m, sm + 15sta, the chromosome arm number NF = 133) composed of three different genomes: *C. taenia*, *C. elongatoides* (n = 24m, sm + 1sta) and *C. tanaitica* (n = 20m, sm + 5sta).

Embryonic and larval development

The pattern of embryonic and larval development of *C. taenia* and polyploid *Cobitis* hybrids was the same (Fig. 1), whereas the duration of the successive stages was longer in polyploids, e.g. hatching of polyploid progeny occurred later (Table 1).

Cleavage phase

An average of 473 (from 247 to 1002) and 443 (from 237 to 634) eggs were obtained from each *C. taenia* and triploid *Cobitis* females, respectively. All eggs were spherical in shape, transparent, with a bright yellow yolk and without an oil globule. First cleavage (Fig. 1a, b) began at the same time post fertilisation (pf) in diploid (1 hour and 40 minutes) and polyploid (1 hour and 45 minutes) embryos. The next cleavages occurred after *c.* 20-30 minutes. Up to the stage of 32 blastomeres, each embryo was formed by a single layer of cells, while afterwards by two cell layers.

Blastula phase

The mid blastula stage (about 1000-cells) was attained at a similar time by diploid (4 hours and 30 minutes pf) and polyploid (5 hours pf) embryos. During this stage the embryo shape changed from elongated to spherical (Fig. 1c, d). At 9-10 hpf, the embryo formed a dome like shape and epiboly began; the blastoderm started to cover the surface of the yolk.

Gastrula phase

In *C. taenia*, at 10 h 40 min. pf, the blastoderm covered half of the surface of the yolksac (50% epiboly), indicating the stage of gastrula. Throughout the margin of the blastoderm, a thickening called the germ ring occurred; at this time epiboly was temporarily stopped. The embryonic shield was formed along the germ ring. Afterwards epiboly was continued and at 15 hours pf the blastoderm completely covered the yolk cell (100% epiboly). Gastrulation in polyploid embryos proceeded in the same mode as in diploids but took evidently longer, from 14 to 18 hpf (on average) (Table 1, Fig. 1e, f).

Segmentation phase

During this period the somites of all embryos were developing, primary organs differentiated from germ layers, the tail bud became more visible

Table 1

Time (hours.minutes) of embryonic developmental stages and hatching of diploid and polyploid *Cobitis* progeny

Parents female x male	Stage and time after fertilization (mean time and range)					
	Cleavage			Gastrulation		Hatching
	2 cell stage	32 cell stage	Blastula (c. 1000 cells)	50% epiboly	Blastopore closed	
C. taenia x *C. taenia*	1.40 (1.30-1.45)	3.20 (3.00-3.30)	4.50 (4.30-5.20)	10.40 (10.00-11.20)	15.00 (14-16)	50.00 (42-59)
3n *Cobitis* x *C. taenia*	1.45 (1.30-2.00)	3.30 (3.00-3.45)	5.00 (4.00-5.30)	14.00 (12.30-19.00)	18.00 (16.00-24.00)	63.00 (5-72)

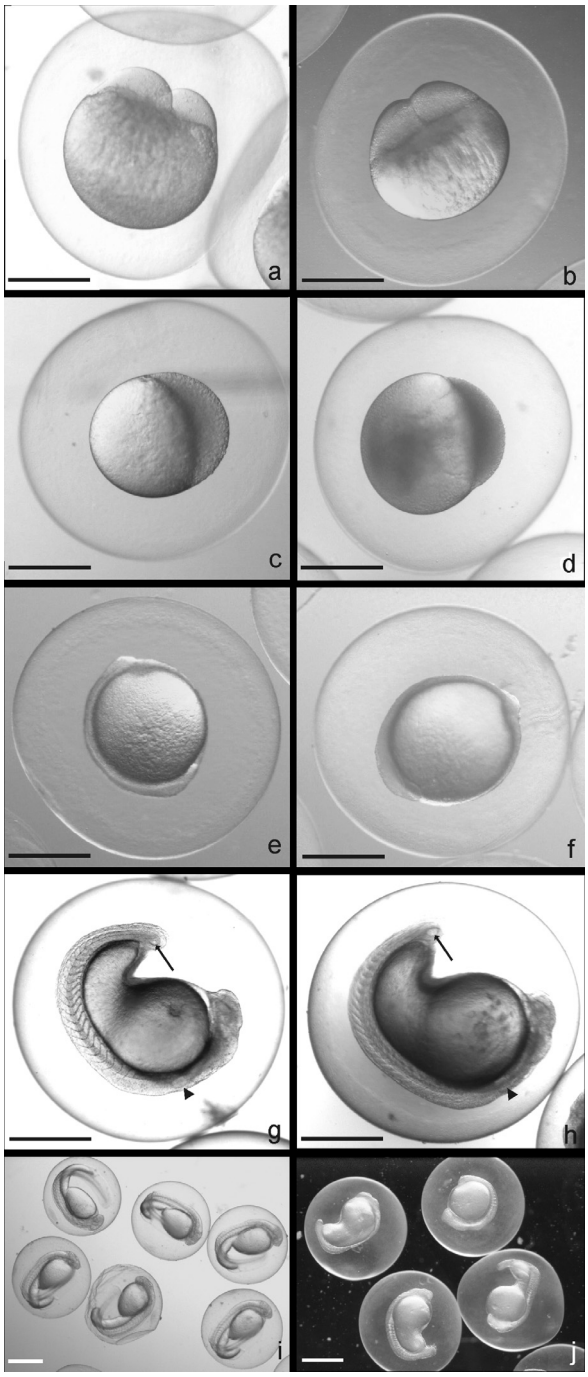

Fig. 1. Embryonic development of *C. taenia* (a, c, e, g, i) and polyploidy of triploid *Cobitis* females (b, d, f, h, j). Two blastomeres (a, b); blastula (b, c); blastopore closure 100% epiboly (e, f); 21 somites, arrow, Kupffer's vesicle, arrowhead, auditory organ (g, h); tail release (i); asynchrony during the segmentation and tail bud stages (j). Bars = 500 μm.

and the embryo elongated. Somite development was directed from the trunk to the tail; the number of somites indicated the developmental stage. Somite formation began at 16 hpf in *C. taenia* embryos and at 21 hpf in polyploid ones. At the stage of 14 somites several distinctive swellings (Fig. 1g, h), termed neuromeres, were observed. The first three neuromeres localized in the anterior part of the embryo corresponding to the brain rudiment.

The visual and auditory (with otoliths) organs appeared (Fig. 1g, h), visible in the anterior part of the embryo. The first body movements were also observed. The tail bud began to develop in the posterior part of the body, and the Kupffer's vesicle was protruding at its base (Fig. 1g, h). At the end of this stage a clear decrease of the yolk amount and the separation of the tail region (Fig. 1i) was observed; anteriorly of the yolk sac a beating heart became visible; the anus was visible posterior to the yolk-extension.

In contrast to *C. taenia*, asynchrony of development was observed in polyploid embryos, mainly during the segmentation and tail bud stages. Simultaneously some of the embryos attained the stage of 14 somites, whereas some others attained the stage of tail bud (Fig. 1j). Almost all of the diploid embryos developed synchronically.

Hatching

Hatching occurred on the third day after fertilization. The average hatching time of polyploid (63 hpf) was statistically significantly longer ($P<0.05$) than of diploid (50 hpf) larvae (Table 1). Larvae perforated the egg shells by the tails (Fig. 2a). Newly

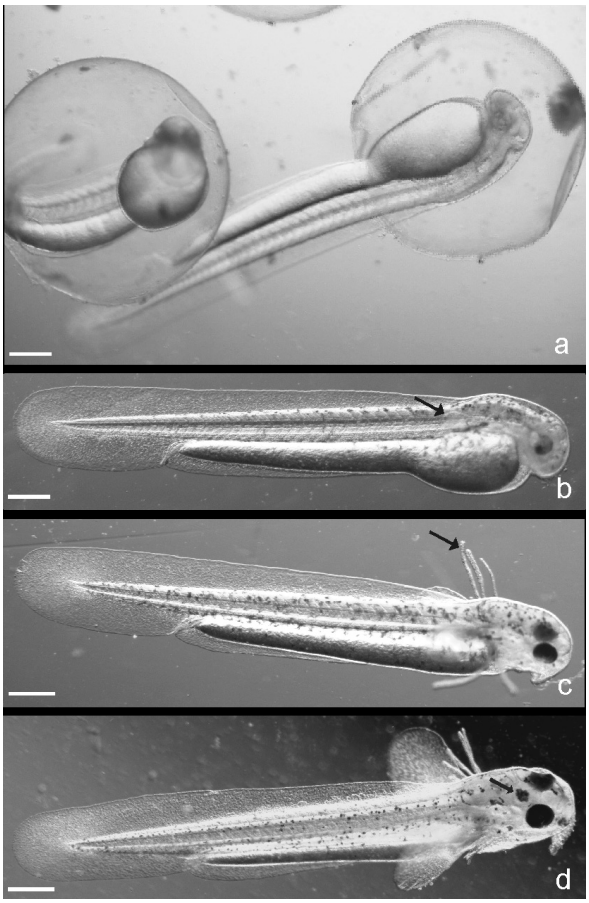

Fig. 2. Larval development of *C. taenia* (a, b, c) and *Cobitis* polyploids (d). Moment of hatching (a); hatched larva, pectoral fin bud, arrow (b); larva 1 dph, external gills, arrow (c); polyploid larva of *Cobitis* at 1 dph, melanophore patch on the head, arrow (d). Bars = 500 μm.

hatched larvae remained at the bottom of the aquarium trembling but did not move. Hatching success of the *C. taenia* progeny (ranged 23-64%, 46% on average, SD±15) did not differ significantly (P>0.05) compared to the polyploid progeny (4-70%, 33.5% on average, SD±27).

Many organs completed their development during this period of morphogenesis. The newly hatched larvae of all embryos contained a large amount of yolk and *c.* forty myomeres. The anterior part of the body was covered with a few melanophores, clearly pigmented eyes; blood circulation and the gut were visible. Dorsal and ventral finfolds developed and pectoral fin buds were visible (Fig. 2b).

At one dph four pairs of external filamentous gills and small pectoral fins were observed in all (diploid and polyploid) larvae and numerous melanophores appeared over their entire bodies (Fig. 2c). However the polyploid larvae had more pigmented bodies, especially in the head region, than diploid ones, and their melanophores formed a large, clear patch on the head, just behind the eyes (Fig. 2d). Moreover, the polyploid larvae were characterized by a high level of deformities concerning *c.* 23% on average. A shortened and C-shaped body, hypertrophy of the pericardium and lack of external gills (Fig. 3a, b) were the most frequently observed deformities. A significantly lower (P<0.05) number of diploid larvae with deformities, *c.* 1%, was counted at the same time of development.

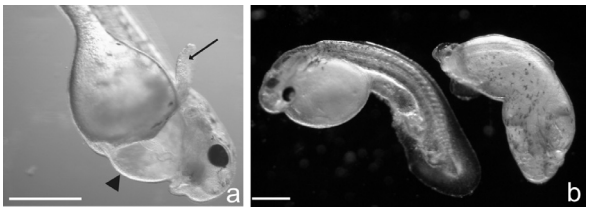

Fig. 3. Deformities in larvae of triploid *Cobitis* females. Hypertrophy of the pericardium, arrowhead, pectoral fin bud, arrow (a); C-shaped body (b). Bars = 500 μm.

At three dph the yolk sac decreased in size and the larvae acquired could actively swim. During this time formation of the mouth-barbell commenced. Four dph larvae began to actively feed exogenously eating the external food. On the eleventh dph the dorsal fin anlage appeared, the first caudal fin rays were distinguished and the external gill filaments shortened and then were covered by the operculum.

The survival rate of larvae at 14 dph fluctuated from 70 to 98% (mean 85%, SD±13) in diploids and from 0 to 100% (mean 75%, SD±38) in polyploids. Seven days later, at 21 dph the mean survival rate of diploid larvae reached 71% (range 31-95%, SD±27) and was still not significantly different (P>0.05) from that of polyploids – 53% (range 0-90%, SD±33).

Post embryonic skeletal development

The successive stages of skeletal development of *C. taenia* and polyploid *Cobitis* were the same (Fig. 4). At one dph in the cleared and stained *C. taenia* and polyploid larvae the notochord was visible as a straight rod extending through the entire length of the body (Fig. 4a, b). Simultaneously in the neurocranium some cartilaginous elements viz. the otic capsule, ethmoid plate and trabeculae cranii (visible between the eyes) appeared. The splanchnocranium consisted of Meckel's cartilage and the pectoral fin buds were located on the lateral body sides (Fig. 4a).

During subsequent days the skeleton of diploid and polyploid larvae developed correctly and no deformations were observed. In all three dph larvae the head skeleton was entirely cartilaginous. In the neurocranium, the paired trabeculae cranii were well developed; the taenia marginalis posterior (orbital cartilage bar paired bilaterally) and the paired cartilaginous parachordals located laterally to the anterior end of the notochord were noticed. Anteromedially the ethmoid plate was formed. In the pharyngeal arches the ceratohyal cartilage and ceratobranchial cartilages 1-4 appeared. The mandibular arch was still composed of the paired Meckel's cartilage (Fig. 4c). The posterior end of the notochord was slightly bent upward and a concentration of cartilage was observed beneath. The pectoral fins and external gill filaments were visible (Fig. 4c). A slender and long cleithrum in the pectoral girdle was the first bony element (Fig. 4d). The ossification of the basioccipital articulatory process, two opercula, two branchyostegal rays and the 4. pharyngobranchial with visible teeth was recorded.

On 5 dph larvae the first rays (lepidotrichia) of the dorsal and anal fins began to form in the finfolds (Fig. 4e). At this time some deformations of the polyploid larvae were observed such as the appearance of an additional eye situated anteriorly between two normally developed eyes and two distinct notochords (Fig. 4f).

On 6 dph larvae the cartilaginous auditory capsule and hyosymplectic cartilage were well developed. The middle part of the ceratohyal and Meckel's cartilages started to ossify, and the developed pharyngeal teeth were clearly visible. The first centra of vertebrae started to develop and ossify in the anterior part of the notochord. In the splanchnocranium the subopercule and three branchiostegal rays were present.

At 11 dph the caudal fin skeleton started to develop in both diploid and polyploid larvae: bony rings of the compound centrum (consisting of a fu-

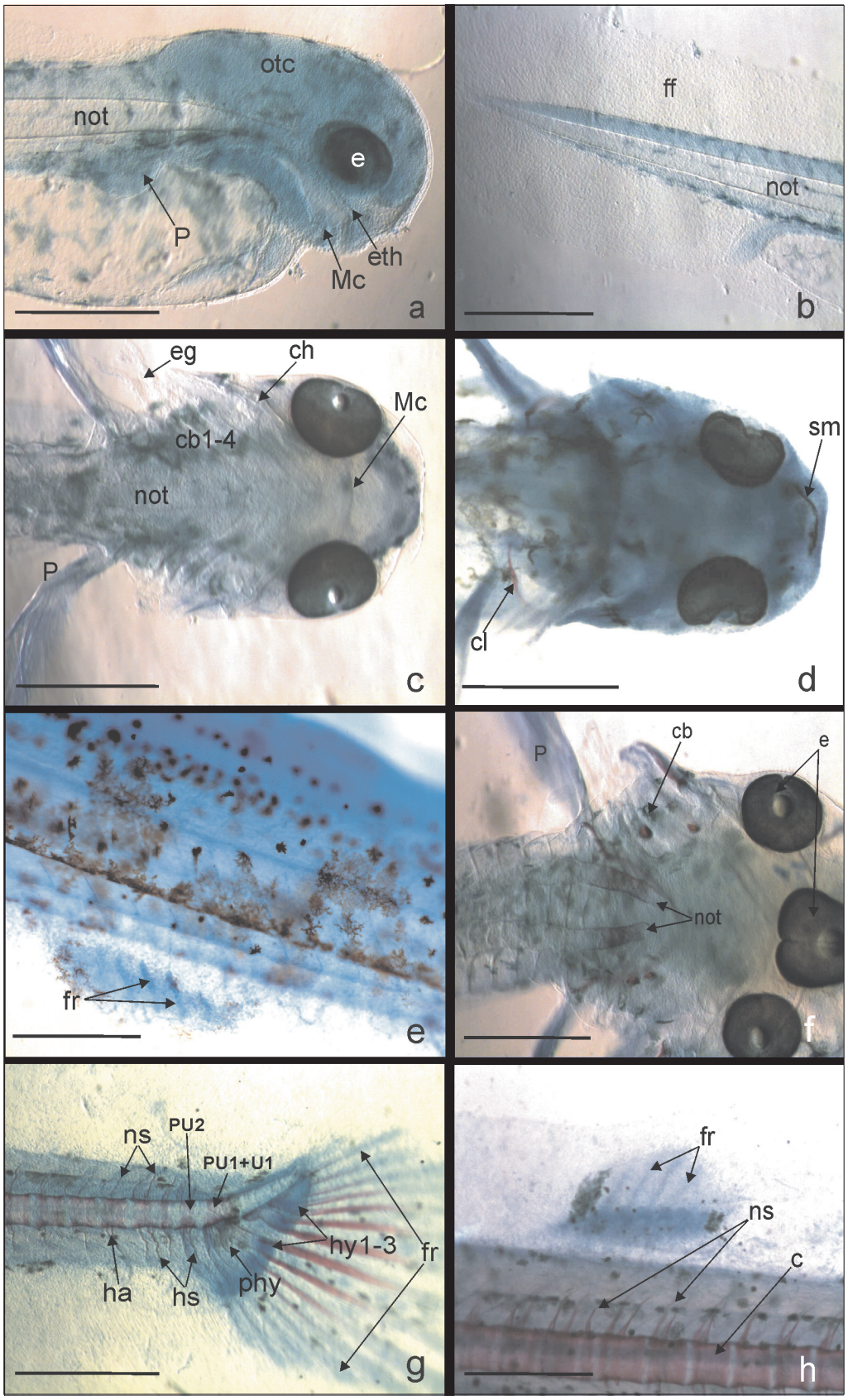

Fig. 4. Skeletal development of *C. taenia* (a, b, e) and *Cobitis* polyploid larvae (c, d, f, g, h). One day post hatching, lateral views (a, b); 3 dph, ventral view (c) dorsal view (d); 5 dph, lateral view (e), dorsal view (f); 11 dph, lateral view (g, h). Abbreviations: c – centrum, cb 1-4 – ceratobranchials, ch – ceratohyale, cl – cleithrum, e – eye, eg – external gills, eth – ethmoid plate, ff – fin fold, fr – fin rays, ha – haemal arch, hs – haemal spine, hy – hypurals, Mc – Meckel's cartilage, not – notochord, ns – neural spine, otc – otic capsulae, P – pectoral fin, phy – parhypural, PU2 – pleural centrum 2, PU1+U1 – compound caudal centrum (1 pleural centrum and 1 ural centrum), sm – supramandibular. Bars = 50 µm.

sion between pleural PU1 and ural U1 centrum) as well as the PU2 centrum (a fusion between the second pleural PU2 and ural U2 centrum) associated with ventral elements, the haemal spine, the parhypural, (1-4) hypurals and (10-16) lepidotrichia, of which 4-10 middle rays were partially ossified (Fig. 4g). In the anterior part of the axial skeleton the Webberian apparatus was visible. In the cranium the following elements were partially ossified: hyosymplectic cartilage, ceratohyal cartilage and ceratobranchials, pharyngeal teeth, palatoquadrate, parasphenoid and entopterygoid. A few larvae already had 5 or 8 visible rays in the dorsal fin (Fig. 4h). Development of anal fin radials (pterygophores) began, but there was no pelvic fin bud visible.

At eleven dph, polyploid larvae exhibited the same elements of the skeleton as diploid larvae, but were characterized by more types of malformations. In the case of diploid larvae deformations in vertebrae were found such as twisted and bifurcated neural and haemal spines. Apart from these malformations (similar as in diploids), some polyploid larvae had supernumerary haemal and neural elements on PU2 (Fig. 4g, h), and nearly half of them had from 1 to 3 twisted ribs.

Discussion

The developmental pattern of the analysed larvae of different ploidy levels was the same, but the rate of development among polyploids was variable in comparison with the balanced rate of diploids, i.e. the diploid progeny developed synchronically whereas polyploid progeny showed asynchrony in development among individuals. Moreover, deformities were more frequent among polyploid than diploid larvae.

In loaches the mitotic division of the zygote (cleavage) is meroblastic, which is typical for other Teleostei. The number of somites is a characteristic feature of embryos that is used to determine the stage of development, and the final number of myomeres is species specific. Hatchlings of species of the genus *Cobitis* are characterized by about 40 myomeres, e.g. *C. taenia* and their hybrids have 40 (present data), *C. takatsuensis* has 37 (SHIMIZU *et al.* 1998) and *C. melanoleuca* has 44 (PAVLOV *et al.* 2004). In both diploid and polyploid embryos in this study, the Kupffer's vesicle, a structure located between the notochord and periblast, was indentified during somitogenesis. Kupffer's vesicle cells control left-right development of brain, heart and gut; this structure was named a transient embryonic "organ of asymmetry" that regulates the earliest known step in left-right axis specification in teleosts (ESSNER *et al.* 2005).

During hatching the fish embryo comes out from eggs mainly head first or tail first. No correlation was seen between hatching orientation and egg diameter or newly hatched larva length (KORWIN-KOSSAKOWSKI 2012). Hatching by tail first was also reported in many other loaches, e.g. in *Misgurnus fossilis* (KRÓL *et al.* 2010) and *I. koreensis* (KO *et al.* 2012) as well as in other species (see review KORWIN-KOSSAKOWSKI 2012). Therefore hatching orientation seems not to be taxonomically informative at the genus or family level. Temperature is emphasized as one of the most important factors affecting the rate of development and growth. The average hatching time (at 24°C) of *C. taenia* and polyploid *Cobitis* larvae was noted at 50 hpf and 63 hpf, respectively. This is similar to *M. angullicaudatus* for which hatching took place at 48 hpf at 20°C (FUJIMOTO *et al.* 2006).

As in other loaches from the genus *Cobitis* (SHIMIZU *et al.* 1998; BOHLEN 2000; KUJAWA *et al.* 2002), *Misgurnus* (SHIMIZU *et al.* 1998; FUJIMOTO *et al.* 2006) and *Iksookimia* (KO *et al.* 2012), the investigated larvae are characterized by long external gill filaments adapting them to enhance oxygen uptake together with vascularized finfolds (BOHLEN 2000).

Successive stages of embryonic and larval development of both *C. taenia* and *Cobitis* polyploids were the same, but the main distinction relates to the duration of development; polyploids developed slowly due to a significant inverse correlation between genome size and developmental (growth) rate (among others: GREGORY 2002).

The second distinction applies to a relatively large number of developmental deformations of polyploid larvae observed similarly in artificially induced fish polyploids (TIWARY *et al.* 2004).

Polyploid larvae examined in the present study may represent two different ploidy levels. Previous genetic studies documented that the progeny of triploid *Cobitis* females was composed of both triploid and tetraploid individuals from gynogenetic and bisexual reproduction, respectively (JUCHNO *et al.* 2014). The tetraploids originated because some of the eggs of 3n females were fertilized by the sperm of *C. taenia* whereas other eggs developed gynogenetically (stimulated by the sperm). It has been shown that tetraploid progeny are characterized by higher mortality in comparison with triploid ones. Thus the observed deformations may concern the tetraploid larvae which died within the first month of life (JUCHNO *et al.* 2014). Surprisingly, the survival rate at 14 and 21 dph of diploid and polyploid larvae was similar. So, most of the observed deformations of polyploid larvae were not lethal and they survived in stable laboratory conditions, without the pressure of predators. The number of tetraploid larvae of triploid *Cobitis* fe-

males decreased significantly at the age of one month and more (JUCHNO *et al.* 2014). Therefore, deformed larvae subsequently died because in older offspring malformations were not observed (unpublished). The inequality observed in polyploid larvae and embryos may be connected with their ploidy level and/or genome composition. The genome of triploid *Cobitis* females from Bug River is composed of three species: *C. taenia*, *C. elongatoides,* and *C. tanaitica*/*taurica* (JANKO *et al.* 2007, 2012; JUCHNO *et al.* 2014; present study). We hypothesised that the deformed polyploid progeny (23%) of triploid *Cobitis* females may result at least in part from their "not adapted genomic composition" so they die and are not further transmitted. However, this process does not stop the clonally originated progeny of triploid *Cobitis* females from growing up and finally dominating (reaching up to 95%) among the *Cobitis* in diploid-polyploid populations (BOROŃ 2003; JUCHNO *et al.* 2007, 2014).

The zebrafish *D. rerio* (Cypriniformes) has become a widely used model organism because its general developmental pattern is similar to all bony fish. Thus, its embryological development is considered to be representative for teleosts (METSCHER & AHLBERG 1999). The postembryonic development of the skeleton in the larvae examined herein reflected the pattern for *D. rerio* as well as that observed in other species of Cypriniformes (CUBBAGE & MABEE 1996; BIRD & MABEE 2003; PARICHY *et al.* 2009). One day after hatching all larvae had only some cartilage elements of the chondrocranium, the straight notochord and the pectoral fin buds. The sole exception concerned the development of a cleithrum in the pectoral girdle as the first bone element. In most Teleostei species, the caudal fin develops as the first bone element among the median fins, followed by the bone structure of anal and then dorsal fin radials and fin rays, and after that the paired fins develop (BIRD & MABEE 2003).

This sequence of ossification was observed in zebrafish and in *M. anguillicaudatus* (ICHIYANAGI & FUJITA 1995), among others. The notochord in the latter species began to flex beneath its posterior end at 5 dph, and at the same time a cartilaginous second hypural was present. In analyzed polyploid larvae the flexion of the notochord was observed earlier, at 3 dph.

Two separate centres of development and ossification were found within the axial skeleton *viz*. the Weberian region and the caudal fin, as is common among Ostariophysi (CUBBAGE & MABEE 1996; BIRD & MABEE 2003). During the early skull development of the analyzed larvae, the trabeculae cranii appeared as the first visible cartilaginous structures within the chondrocranium. The first bones which appeared in the osteocranium consisted of the opercula, similarly to the sequence of bone appearance noted in cyprinids, the zebrafish (CUBBAGE & MABEE 1996) and *Barbus barbus* (VANDERVALLE *et al.* 1992).

Similarly as in the investigated larvae of *Cobitis*, tiny fin buds of pectoral fins in the zebrafish were observed at the end of the first dph (GRANDEL & SCHULTE-MERKER 1998). These fins develop in two phases, the larval phase which is maintained during the first two weeks of life, and the adult fins. In the larvae of the present study the first anatomical ossified element was the pectoral fin girdle (cleithrum) followed by the axial skeleton: the caudal fin, the vertebral column, the dorsal and anal fins and lastly the pelvic fins. The latter were not observed within 11 days of development. Similarly in the zebrafish, the pelvic fin buds begin to develop during the third week post fertilization (18 dpf) and the skeleton was formed completely after four weeks (29 dpf) (GRANDEL & SCHULTE-MERKER 1998). The chondrification and ossification sequence of the skeleton in cobitid taxa merits further study.

Skeletal deformities are relatively well known in fish but most of the data concern the progeny of commercially important species (tench *Tinca tinca,* grass carp *Ctenopharyngodon idella,* Atlantic cod *Gadus morhua*) and also species artificially induced to reproduction (common carp *Cyprinus carpio,* Atlantic salmon *Salmo salar,* rainbow trout *Oncorhynchus mykiss*) (BENFEY 1999; FELIP *et al*. 2001; TIWARY *et al*. 2004; SFAKIANAKIS *et al.* 2006; MAXIME 2008; PIFERRER *et al.* 2009; FJELLDAL & HANSEN 2010; BOGLIONE *et al.* 2013; FRASER *et al.* 2013).

Some data indicate that induced triploids are sterile and grow faster than diploids, but their larvae are characterized by a higher incidence of deformities and a lower survival rate in comparison with diploid ones (TIWARY *et al.* 2004; PIFERRER *et al.* 2009; FJELLDAL & HANSEN 2010; FRASER *et al.* 2013). Interestingly, the larvae of triploid *Cobitis* females were longer than those of *C. taenia* but only during the first two weeks of life (JUCHNO *et al.* 2013). Afterwards, their length did not significantly differ from that of diploids.

However, such deformities are generally rare in wild populations (BOGLIONE *et al.* 2001, 2013). Their occurrence is attributed to a variety of causative factors acting strongly during the early life stages, usually with common symptoms and probably with cooperative effects (SFAKIANAKIS *et al.* 2006).

Deformities observed in both diploid and polyploid larvae of *Cobitis* taxa appeared along with the proceedings in ossification. In diploids deformities of the neural and/or haemal arches were

observed, while in allopolyploids numerous deformities concerning the whole body were additionally found. The development of polyploid *Cobitis* larvae seems to be influenced by their genome composition (of three different species) because no deformities were observed in the caudal skeleton development of laboratory reared related species *M. anguillicaudatus* of polyploid origin (ICHIYANAGI & FUJITA 1995). However in allopolyploidy, it is difficult to separate the effects of polyploidy with those of hybridization (BENFEY 1999).

Our results showed that although diploid and polyploid progeny obtained from *C. taenia* and allotriploid *Cobitis* females, respectively, show the same developmental pattern, but they differ in the length of embryonic development which takes significantly more time in polyploids. Significantly more individuals of polyploid progeny demonstrated asynchrony during early development and deformities during larval development. However their hatching success and survival rate was similar to that of diploid progeny.

The presented results expand our knowledge of the functional biology of the spined loach *C. taenia* and its allotriploid hybrids and contribute to a better understanding of some aspects of their early life history. These data show how a larger genome influences the early development and skeletal formation of naturally occurring diploid and polyploid taxa. We conclude that the domination of triploid *Cobitis* females in diploid-polyploid populations does not seem to be connected directly with embryonic and larval development. The presented data contribute to understanding the true extent and role of polyploidy in fishes.

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