Research Article

Taxonomic studies of *Piaractus brachypomus* (Cuvier, 1818) (Serrasalmidae): a non-native species collected from Polish water bodies and laboratory culture

Beata Więcaszek[1,*], Jarosław Dąbrowski[2], Remigiusz Panicz[3], Sławomir Keszka[4], Angelika Linowska[1] and Adam Brysiewicz[2]

[1]*West Pomeranian University of Technology in Szczecin, Dep. of Hydrobiology, Ichthyology and Biotechnology of Breeding, 4, K. Krolewicza st., 71-550 Szczecin, Poland*

[2]*Institute of Technology and Life Sciences - National Research Institute, Hrabska Avenue3, 05-090 Falenty, Poland*

[3]*West Pomeranian University of Technology, Szczecin, Dep. of Meat Sciences, Faculty of Food Science and Fisheries, 4, K. Krolewicza st., 71-550 Szczecin, Poland*

[4]*Border Veterinary Inspection Post in the Port of Szczecin, Poland*

Author e-mails: *bwiecaszek@zut.edu.pl (BW), angelika.linowska@zut.edu.pl (AL), j.dabrowski@itp.edu.pl (JD), a.brysiewicz@itp.edu.pl (AB), rpanicz@zut.edu.pl (RP), keszka@gazeta.pl (SK)*

*Corresponding author

Abstract

Piaractus brachypomus is highly valued as an aquaculture and ornamental fish species. However, it is frequently considered as a non-native or invasive species. In Polish waters *P. brachypomus* is an incidental non-native species, most likely released by aquarists, and it is unable to overwinter or reproduce. Outside its native distributional range, this species has been recorded nearly world-wide. In regions where exist it is often confused with other species such as *Piaractus mesopotamicus* and *Piaractus orinoquensis*, possibly with *Colossoma macropomum* and their hybrids, and *Pygocentrus nattereri*. In this study, we provide a detailed taxonomic description and the most useful discriminative features for the species, of five specimens of *P. brachypomus* caught in water bodies in northwestern Poland (in 2002–2010), and a sample of 11 specimens obtained from laboratory culture (2012–2019) that originated from South America. The examinations included describing metric and meristic characters, the neurocranium structure, the dentition formula, parasite fauna, and species identification based on a mitochondrial COI sequence. The phylogenetic tree inferred using maximum likelihood showed that pirapitinga samples were nested within the Amazon Basin clade of *P. brachypomus*. This is the first molecular study in Europe and first detailed morphometric study (including the neurocranium) of this species from a non-native area of its distribution. Characters such as dentition can be used to discriminate among the genera *Colossoma*, *Piaractus* and *Pygocentrus*, while meristic characters such as lateral line scale counts, numbers of scale rows above and below the lateral line, pectoral and pelvic fin rays counts, and ventral-keel spine counts can be used to discriminate among species within the genus *Piaractus*. Nevertheless, morphometric characters do not seem to be an appropriate tool to distinguish closely related species in the genus *Piaractus*.

Key words: pirapitinga, molecular studies, non-native occurrence, morphometrics, dentition formula, neurocranium structure

Introduction

Piaractus brachypomus (Cuvier, 1818), pirapitinga, attains a total length of 88 cm (IGFA 2001) and weights of up to 25 kg (Baensch and Riehl 1985). Adult pirapitinga have few predators because of their large size and

powerful teeth (Lovshin 1995). Until recently, pirapitinga (red-bellied pacu, white tambaqui, or caranha) was considered a single tropical panmictic species lacking meristic differences among fishes from the Amazon and Orinoco river basins and undertaking extensive spawning migrations. Its distribution was noted to include Bolivia, Brazil, Colombia, Peru, and Venezuela where it inhabited large flooded rivers and lakes (Machado-Allison 1983; Jégu 2003). Géry (1985) showed the extensive range of intraspecific phenotypic variability, which raised the possibility of cryptic species, and this author discussed the taxonomy and distribution of *P. brachypomus* and small-scaled pacu *Piaractus mesopotamicus* (Holmberg, 1887). However, despite these observations, all subsequent taxonomic and molecular studies (e.g., Ortí et al. 2008; Oliveira et al. 2011) and biological inventories (e.g., Agudelo Cordoba et al. 2011; Lasso and Sánchez-Duarte 2011) followed the taxonomy and distribution of *P. brachypomus* proposed by Machado-Allison (1983). Escobar et al. (2015) were the first to determine that *P. brachypomus* of the Orinoco and the Amazon basins actually represented distinct, independent evolutionarily lineages, and, therefore, different species, and *Piaractus orinoquensis* Escobar, Ota, Machado-Allison, Farias & Hrbek, 2019, is a new species of serrasalmid fish described from the Orinoco River Basin (Escobar et al. 2019). Jorge et al. (2018) confirmed that the distribution of *P. brachypomus* was restricted to the Amazon River Basin. This species, or, more precisely the *Piaractus* cf. *brachypomus*, was also noted in many countries and regions (at least 14) outside its native distributional range, including in the South, Central, and North Americas, Oceania, Europe, Australia and Asia (Correa et al. 2014). Individuals are released by aquarists, or unintentionally, from aquaculture stocks (Katwate et al. 2012).

Pirapitinga is highly valued as an aquaculture fish species (Katwate et al. 2012), and it is considered to be one of the main native species used in aquaculture production in South America (Saint-Paul 2017; Jorge et al. 2018; Escobar at al. 2019). The cultivation of pirapitinga and tambaqui (gamitana) *Colossoma macropomum* (Cuvier, 1816), species of the group known as pacus in Brazil, as well as hybrids of them have been bred for many years in the countries of the Orinoco River Basin (Rodriguez et al. 2007). In Brazil, representatives of the Serrasalmidae family, i.e., *C. macropomum*, *P. brachypomus*, *P. mesopotamicus*, and their interspecific hybrids, are native fishes with the largest production in Brazilian aquaculture. *Piaractus brachypomus* can also hybridize with *P. orinoquensis*, but the offspring appears to be sterile (Escobar at al. 2019). This fish group is also widely farmed in other Latin American countries (Colombia, Venezuela, and Cuba) (Hashimoto et al. 2014) and Asian countries including China, Myanmar, Thailand, Vietnam (Honglang 2007; Xiong et al. 2015), Bangladesh, and India (Praveenraj et al. 2014; Roshni et al. 2014). This species was introduced to Malaysian aquaculture from Taiwan in 1984 (FAO 2020). It

was also introduced deliberately into Zarivar Lake (western Iran) in 2016 as a result of unmanaged aquaculture with the aim of improving local fisheries (Zarei and Rajabi-Maham 2017), and in Papua New Guinea where a major introduction of *P. brachypomus* took place in 1990 as part of a fish stock enhancement project (Coates 1997; Correa et al. 2014). In general, representatives of the genera *Piaractus* and *Colossoma* and their hybrids are economically important as their culture potential is even higher (Campos-Baca and Kohler 2005; Kumar et al. 2018).

Pirapitinga, which is also valued as an aquarium fish species, has been introduced into several countries as an ornamental fish. However, when these fish reach large sizes in aquarium culture, they are frequently released into water bodies and become pests in non-native environments (Więcaszek et al. 2009). The first record of *P. brachypomus* and the first representative of Serrasalmidae reported in waters of central Europe was described in 2002 in Poland (Boeger et al. 2002; Więcaszek et al. 2007). The most recent records of it in Poland (seven individuals) date from August 2019 (representatives of *Piaractus*, not precisely identified) and one individual identified as *C. macropomum* (50 cm total length, 3150 g weight) was caught in an artificial water body by anglers in September 2020 (own unpublished data). The trade of pirapitinga has been restricted by the Minister of the Environment in Poland since 2014 as it is considered an alien species that poses a potentially negative impact on the native environment. It is also included in the Nordic-Baltic Network on Invasive Species in Poland, as a non-invasive, alien species (Więcaszek et al. 2016).

In Europe, incidental finds of individual pirapitinga have been recorded in Slovakia, Spain and Croatia, and they are assumed to have been released by aquarists. A live pirapitinga was also caught in the Øresund (Baltic Sea, salinity of approximately 13) in 2013 (Leunda 2010; Więcaszek et al. 2016). Outside Europe, other introductions of *P. brachypomus* as an aquarium fish took place in the Philippines (Cagauan 2007), the Canadian Province of Ontario (Coad et al. 1995), and in Malaysia, where the culture of this species is regulated to prevent accidental introduction into water bodies; hence, the culture is small-scale and mainly for ornamental purposes (Kechik 1995). In the U.S. individual specimens have been caught in 20 states since the 1960s, probably through aquarium releases or fish farm escapes (Marchetti et al. 2004; Di Santo et al. 2018). In 2020 pirapitinga was recorded in U.S. in 12 locations (Nico et al. 2021). Recently it was also recorded in Indian wetlands (Tiknaik et al. 2019), as well in a lake in Guatemala, where the most likely source of introduction was the local aquarium trade (Elias et al. 2018).

Pirapitinga is considered either an alien species or invasive alien species (IAS) depending on the area of non-native occurrence. According to Minister of Environment of Canada (Environment Canada 2012) it is an alien species in temperate zone, but it becomes an IAS in subtropical or

tropical regions where overwintering and breeding are. For example, in Australia (Queensland) *P. brachypomus* is on the Restricted Noxious Fish List (Queensland Government 2020). However, progressive global warming could increase the distribution of nonindigenous occurrences of *P. brachypomus* as more effective overwintering and breeding possibilities become available.

In many countries, individuals of *P. brachypomus* caught in open waters have been misidentified and reported as red piranha, *Pygocentrus nattereri* Kner, 1858, usually in the juvenile stage. This species is also often confused with other species, such as *C. macropomum*, *P. mesopotamicus*, and recently with *P. orinoquensis* and their hybrids (Escobar et al. 2019; Nico et al. 2021). Some individuals caught in the wild were identified only as *Piaractus* sp. or, for example as *Piaractus* cf. *P. brachypomus*, especially in Asian countries (Praveenraj et al. 2014), but also in the U.S. (Nico et al. 2021), Canada (Hanke et al. 2006), and Russia (Podushkov 2016). According to Singh (2018), morphological information, characteristics, and the biology of pacus found in India have yet to be scientifically validated.

Only single specimens from non-native distribution have been described to date, and no molecular studies have been conducted, except on one specimen from India described in Tiknaik et al. (2019). The neurocranium and dentition structures have also not been well documented to date and only a few characters have been described. A recent morphological description of *P. brachypomus* might also have identified a new species *P. orinoquensis* (Escobar et al. 2019).

Since the proper identification of non-native species is crucial to understanding bio-invasion processes, the objective of the present paper was to provide a detailed morphological description, and the most important discriminative features for the species, comprising neurocranium characters, the dentition formula, parasite fauna, and the molecular identification of *P. brachypomus* individuals collected from water bodies and from the aquarium trade in Poland that originated from South America.

Materials and methods

A total of 16 specimens of *P. brachypomus* were examined. Five individuals were collected in West Pomerania water bodies (northwestern Poland) in 2002–10, and they measured 15.3–37.0 cm in total length *TL* and 65.5–1,169 g in weight *W*. Catch locations and periods were described in detail in the paper by Więcaszek et al. (2016). The remaining eleven specimens were from aquarium culture obtained in 2012–19 (25.1–42.3 cm *TL*, 331.5–1,650 g *W*).

Molecular study

Samples for molecular studies were collected from the muscle tissues. DNA isolation was performed with a High Pure PCR Template Preparation Kit

(Roche Life Science, Mannheim, Germany) following the manufacturer's instructions. The qualitative and quantitative assessments of the isolates were conducted by electrophoresis in 1.5% agarose gel followed by spectrophotometric measurements using a NanoDrop 2000 instrument (Thermo Scientific). For all the samples investigated, the PCR amplification of the COI gene was amplified with VF1d_t1 and VR1d_t1 primers as described in Ivanova et al. (2007). The amplification of the selected region was conducted using the following cycles: 1 step of 5 min at 94 °C followed by 35 cycles at 94 °C for 30 s, 56 °C for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 10 min. The PCR reaction was conducted on a Mastercycler (Eppendorf, Hamburg, Germany) with a GoTaq PCR kit (Promega), i.e., in a 25-μl reaction volume containing 5 μl of 5X Colorless GoTaq Flexi Buffer, 2.5 mM of $MgCl_2$, 0.5 μl of each primer at 10 pmol/μl, 0.5 μl of 0.2 mM of dNTP, 200 ng of total DNA and 1 U of GoTaq polymerase. Amplification results were assessed by separating the PCR products analyzed on 1.5% agarose gel. Purified PCR products were sequenced bidirectionally with direct Sanger sequencing by Genomed (Warsaw, Poland) using the same primers as in amplification. The sequences were assembled and aligned with Geneious v.8 (Biomatters) to obtain consensus sequences. Ambiguous base calls were manually annotated using corresponding chromatograms, and sequence identity was determined with a BLASTn search. In the analyses involving the COI gene, we also included sequences of *P. brachypomus* (KP723341 to KP723360) published by Escobar et al. (2015), *P. orinoquensis* (MK395376 to MK395386) published by Escobar et al. (2019), and *P. mesopotamicus* (HQ420833, GU701416), *Colossoma macropomum* (HQ420847), and *Mylossoma duriventre* (Cuvier, 1818) (HM453212) obtained from GenBank in order to determine the Orinoco or the Amazon basin origin of the analyzed *P. brachypomus* samples from this study. A phylogenetic tree was constructed with the Maximum Likelihood method (1000 bootstrap, Kimura 3-parameter model) in MEGA 7.0 software.

Morphometric and meristic studies

Measurements were performed according to methods described in Więcaszek et al. (2009) with marked modifications (Figure 1) that included the following measurements: height of scaled part of adipose fin, postdorsal distance, distance between dorsal and adipose fins, length of lower and upper lobes of caudal fins, and upper jaw length. The following measurements were excluded: dorsal spine's width and height, pectoral spine length, and mandibulary barbel length. Fish body measurements were taken on the left side of the specimens, with an electronic caliper under a Nikon SMZ 1000 electronic microscope with a Lucia Measurement System to the nearest 0.1 cm, while neurocranium measurements were taken to the nearest 0.01 mm. The fish were weighed to the nearest 0.5 g. The *TL–W* relationship of the fish examined was calculated.
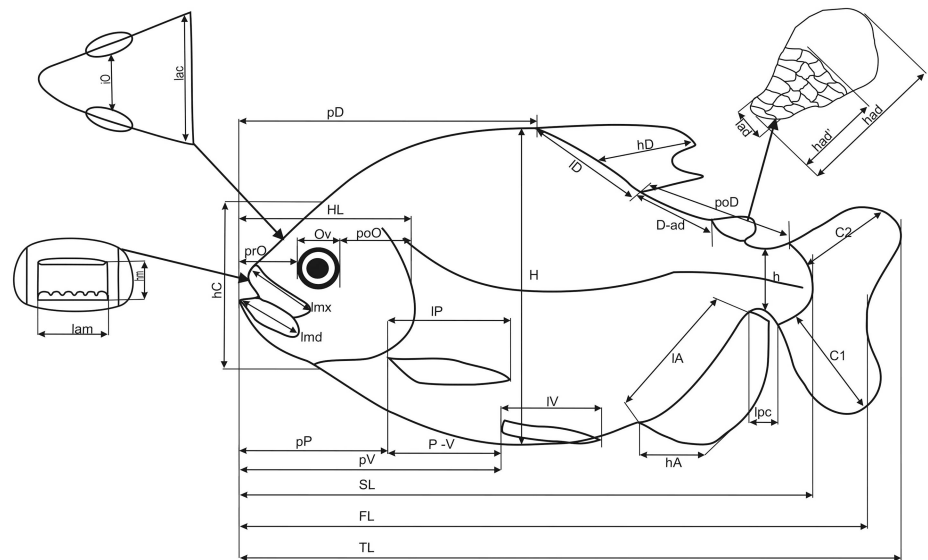
Figure 1. Body measurement pattern of *Piaractus brachypomus* (from Więcaszek et al. 2009) (see Table 1 for abbreviations).

Table 1. Morphometric characters of the *Piaractus brachypomus* body as percentages of standard length *SL*.

Length class	15.0–30.0 cm (N = 8)			30.1–45.0 cm (N = 8)		
Body character	Range	M ± SD	CV	Range	M ± SD	CV
Head length *HL*	27.7–32.3	30.1 ± 1.6	5.3	25.2–31.1	28.1 ± 2.1	7.4
Predorsal distance *pD*	52.4–57.1	54.4 ± 1.6	3.0	50.5–54.6	52.8 ± 1.6	3.1
Postdorsal distance poD	32.6–37.6	34.5 ± 1.9	5.5	34.1–39.4	36.3 ± 1.9	5.1
Maximum height *H*	45.0–47.7	46.5 ± 1.1	2.3	42.4–49.4	46.6 ± 2.3	4.9
Preanal distance *pA*	47.5–74.1	69.2 ± 9.7	14.0	69.5–74.6	72.0 ± 1.7	2.4
Minimum heigth *h*	10.1–11.6	11.1 ± 0.5	4.8	10.2–12.7	11.4 ± 0.8	6.9
Length of caudal peduncle *lpc*	6.1–14.7	9.7 ± 2.8	28.8	6.9–13.5	9.7 ± 2.6	26.4
Length of caudal fin *lC*	17.1–24.3	20.0 ± 2.6	12.9	18.1–28.5	23.2 ± 3.4	14.7
Length of upper lobe C_2	21.7–24.5	23.0 ± 1.2	5.3	18.8–25.1	21.5 ± 1.9	8.8
Length of lower lobe C_1	23.2–27.9	25.7 ± 1.8	6.8	21.9–27.2	23.9 ± 2.1	8.6
Prepectoral length *pP**	23.6–28.7	26.1 ± 2.0	7.5	18.5–28.1	22.2 ± 3.8	17.1
Pectoral fin length lP	14.3–22.6	17.1 ± 3.0	18.0	14.2–17.3	15.8 ± 1.3	8.4
Height of dorsal fin *hD*	16.3–30.2	23.1 ± 5.2	22.3	16.3–30.3	20.7 ± 4.6	22.1
Height of anal fin *hA*	14.3–20.3	17.0 ± 2.2	13.0	12.9–18.5	15.7 ± 2.0	13.0
Length of dorsal fin base *lD*	21.5–23.2	22.3 ± 0.6	2.9	19.1–23.0	21.4 ± 1.5	6.8
Length of anal fin base *lA*	23.4–25.9	24.7 ± 0.8	3.1	23.3–25.8	24.7 ± 0.9	3.6
Distance *P-V*	20.2–24.3	22.5 ± 1.3	5.9	20.6–25.5	23.9 ± 1.8	7.5
Distance *V-A*	21.0–29.0	24.3 ± 3.0	12.4	23.6–27.2	25.4 ± 1.1	4.4
Distance *D-ad*	18.3–20.4	19.0 ± 1.0	6.7	21.5–23.9	22.5 ± 1.4	8.7
Height of adipose fin *had*	4.3–6.8	6.0 ± 0.8	13.9	4.9–6.6	5.6 ± 0.7	12.0
Length of adipose fin base *lad*	2.3–4.0	3.4 ± 0.6	17.8	2.5–4.3	3.1 ± 0.8	25.2
Height of scaled part of adipose fin *had'*	2.8–4.8	3.9 ± 0.8	20.2	3.0–5.2	3.7 ± 0.7	18.9
had' in percentage of *had*%						
Height of scaled part of adipose fin	43.0–73.3	64.8 ± 10.3	15.9	41.2–64.7	55.2 ± 8.9	16.0

* difference statistically significant; p-level < 0.05
Abbreviations: M – mean; SD – standard deviation; CV – coefficient of variation.

The sample was divided into two *TL* classes, according to the fish maturity stage – juvenile up to 30 cm, subadult – above 30 cm TL (Loubens and Panfili 2001): 15.0–30.0 and 30.1–45.0 cm (each class had eight individuals). The analysis comprised 31 metric characters: 22 of the fish body and nine of the head. All characters and their symbols are described in Table 1 (body characters) and Table 2A (head characters). All the lengths

Table 2. *Piaractus brachypomus* head characters as percentages of head length *HL* (A), and neurocranium morphometric characters as percentages of total cranial length *meth-soc* (B).

Length class	15.0–30.0 cm (N = 8)			30.1–45.0 cm(N = 8)		
A. Head character	Range	Mean ± SD	CV	Range	Mean ± SD	CV
Preorbital length *prO*	22.5–28.8	26.1 ± 2.5	9.7	21.1–32.2	28.4 ± 4.0	14.1
Horizontal eye diameter *Oh**	17.7–30.0	22.4 ± 4.2	18.9	16.9–20.7	18.3 ± 1.4	7.4
Length of lower jaw *lmd*	32.1–45.0	38.6 ± 4.4	11.3	33.7–39.0	36.2 ± 2.2	6.1
Length of upper jaw *lmx*	23.9–31.8	27.4 ± 2.6	9.6	26.7–36.7	31.2 ± 3.9	12.6
Width of mouth *lam*	29.0–39.4	34.4 ± 3.6	10.4	28.6–37.8	33.6 ± 2.8	8.4
Postorbital length *poO**	42.5–56.1	49.8 ± 5.3	10.7	51.2–60.0	56.3 ± 3.3	5.8
Head height *hC*	67.2–96.5	77.5 ± 9.5	12.3	65.6–86.7	74.6 ± 7.9	10.6
Head width *lac*	44.3–70.2	52.3 ± 8.7	16.6	44.1–62.2	53.1 ± 7.0	13.1
Interocular distance *iO*	41.8–49.3	45.1 ± 2.5	5.4	41.0–50.00	47.2 ± 3.3	7.0
B. Neurocranium character						
Distance from the anterior part of the vomer to the basiooccipitale bone *v-boc*	47.3–62.1	55.7 ± 6.6	11.9	47.3–62.3	53.9 ± 6.8	12.7
Width of the sphenotic bone *la.spho*	52.3–56.4	53.9 ± 1.6	2.9	50.2–55.0	53.0 ± 2.2	4.2
Width of the lateral ethmoid bone *la.ethl*	40.0–44.2	41.3 ± 1.8	4.3	39.6–45.0	42.4 ± 2.4	5.6
Width of the mesethmoid bone *la.meth*	15.3–17.8	16.7 ± 1.0	6.1	15.4–18.0	16.6 ± 1.1	6.7
Height of the ethmoid lateral bone *h.ethl*	27.0–33.6	29.4 ± 2.7	9.1	27.8–32.4	30.1 ± 2.0	6.7
Heigth of the sphenotic bone *h.spho*	32.6–38.3	34.2 ± 2.5	7.4	32.9–33.9	33.5 ± 0.4	1.3
Height of the supraoccipital bone *h.soc*	51.7–57.7	54.7 ± 2.3	4.1	51.1–56.5	53.8 ± 2.4	4.4
Width of the epiocciptal bone *la.epioc*	30.1–33.2	31.8 ± 1.3	4.1	31.0–32.7	31.73 ± 0.8	2.4
Length of the supraoccipital crest *l.soc**	33.6–40.3	36.7 ± 2.8	7.6	39.4–40.9	40.1 ± 0.6	1.6
Total length of fontanel *l.fon*	36.1–41.1	38.6 ± 1.8	4.7	34.9–41.4	38.0 ± 2.9	7.6
Length of anterior part of fontanel *l.fon$_1$*	11.7–15.7	14.0 ± 1.6	11.3	13.5–14.5	14.0 ± 0.5	3.3
Length of posterior part of fontanel *l.fon$_2$*	15.8–20.7	17.8 ± 1.9	10.5	17.1–18.8	17.8 ± 0.8	4.3
Width of anterior part of fontanel *la.fon$_1$*	7.0–9.2	8.2 ± 0.9	10.6	7.3–8.2	7.7 ± 0.4	5.6
Width of posterior part of fontanel *la.fon$_2$*	4.8–7.0	6.0 ± 0.8	14.1	5.9–7.5	6.7 ± 0.7	10.3
Width of the epiphyseal bar *la.b.ephi*	6.9–10.0	7.9 ± 1.3	16.8	7.5–8.4	7.9 ± 0.4	5.0

* difference statistically significant; p-level < 0.05.
Abbreviations: M – mean; SD – standard deviation; CV – coefficient of variation.

were calculated in cm and calculated into percentages; the body measurements were expressed as proportions of standard length *SL*, while head measurements were expressed as proportions of head length *HL*. Additionally, the height of scaled part of the adipose fin – *had'* was expressed as the proportion of the height of the adipose fin – *had*.

Neurocranium measurements comprised 17 characters: the total cranial length – *meth-soc* (i.e., the distance from the anterior tip of the mesethmoideum to the tip of the supraocciptal crest), the distance from the anterior part of the vomer to the basiooccipitale – *v-boc* (i.e., the bottom length of the neurocranium); the widths of the following bones: the sphenotic – *la.spho*; the lateral ethmoid – *la.ethl*; the mesethmoid – *la.meth*; the epiocciptal – *la.epioc*; the depth of following bones: lateral ethmoid – *h.ethl*; the sphenotic – *h.spho*; the supraoccipital – *h.soc*, and the length of the supraoccipital crest – *l.soc*. In addition, the fontanel was examined in detail: total length – *l.fon*; lengths and widths of the anterior and posterior parts of the fontanel (*l.fon$_1$*, *l.fon$_2$*; *la.fon$_1$*, *la.fon$_2$*, respectively); the width of epiphyseal bar – *la.b.ephi* (Figure 2). All cranial characters were expressed as percentages of cranial length *meth-soc*. The dentition arrangement was analyzed on the premaxillare, maxillare, and dentale bones.
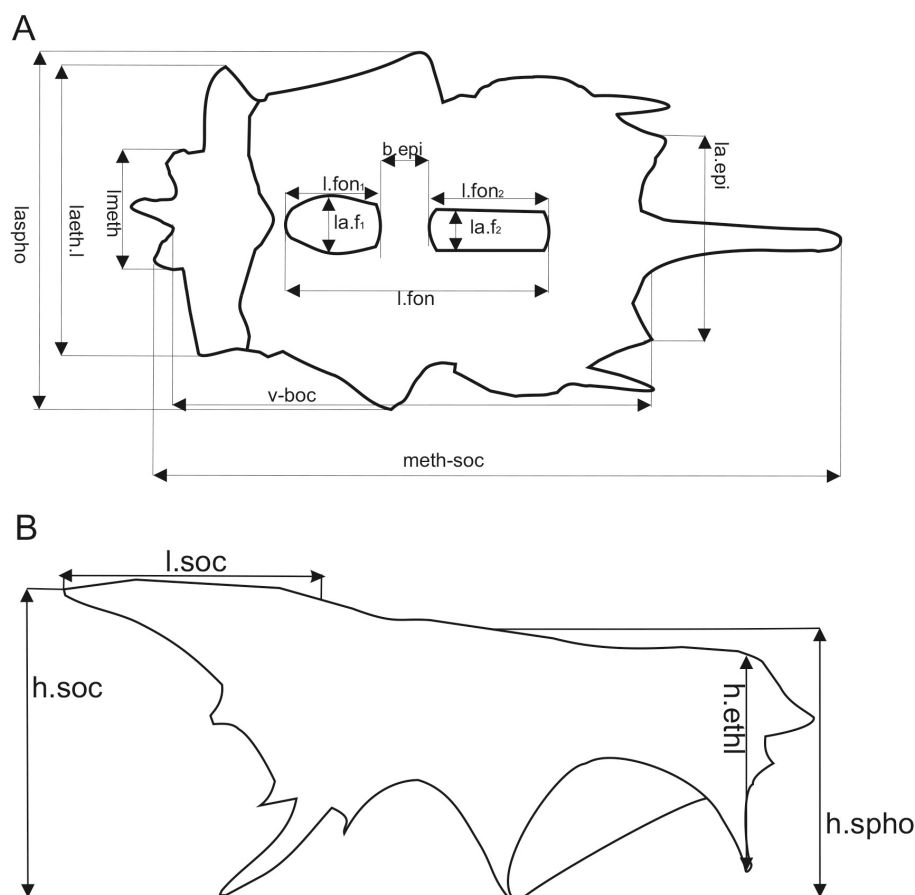
Figure 2. Neurocranium measurement pattern of *Piaractus brachypomus*; A dorsal view; B lateral view (see Table 2 for abbreviations).

Twenty meristic counts were examined: the number of rays (unbranched and branched) in the dorsal *D*, anal *A*, pectoral *P*, and pelvic *V* fins; vertebral count *vt* (including the Weberian apparatus as four elements and the fused PU_1+U_1 as a single element); counts of ventral-keel spines: pre-pelvic spines K_1 (extending to the origin of the pelvic fin but not including the spine lying over the pelvic fin insertion) and post-pelvic spines K_2 (including the spine over the pelvic-fin origin, plus those from the pelvic fin origin to the double pair of spines); total ventral-keel spines count *K*; lateral line scale count (*l.l.*); count of scale rows above and below the lateral line (*l.l.$_a$*, *l.l.$_b$*); count of scale rows on the adipose fin ad_{sr}, anal A_{sr} and caudal C_{sr} fins; the gill-raker count on the first gill-arch in two rows (GR_1, GR_2). The dimensions of lapillus (length and width) was described for specimen of 31.3 cm TL.

Statistical analysis

Statistical analyses were conducted in Statistica v. 13 and in the statistical environment R (packages: rattle, Rcmdr, Rgtk2, ggplot2, dunn.test). We used the Shapiro-Wilk test to check the normality of the data. If the test showed a lack of normality, we used non-parametric tests (the Kruskal-Wallis test and the Dunn test as post-hoc tests), and if the test showed

normality, we used the parametric Student's t-test. Student's t-test was used to verify the hypothesis of the lack of statistically significant differences among the average values of the morphometric characters of the body and head, while the Kruskal-Wallis test was used to verify the hypothesis of the lack of statistically significant differences between the average values of neurocranium characters in the two length classes.

Correlations were calculated among all the metric characters of body, head, neurocranium, and meristics, and standard length *SL*. In addition, the correlations among all neurocranium characters with cranial length *meth-soc* were calculated. For all characters, both meristic and metric, the mean, standard deviation SD and coefficient of variation CV were calculated.

The significance level in all analyses was considered at $p < 0.05$.

Parasitological study

All the fish were subjected to parasitological examination. In the first stage, the fish were examined macroscopically for ectoparasites. Subsequently, nasal and oral cavities and scrapings from the body surface under the pectoral fin were collected and made into wet slides. Finally, the gill arches were isolated and analyzed under a stereomicroscope (Zeiss Stemi DV4) and a microscope (Olympus BX50). Isolated digestive tubes and viscera preparations were also reviewed under the microscope and stereomicroscope.

Results

Molecular study

Sequence comparisons demonstrated that specimens of *P. brachypomus* had homogeneous COI regions (denoted as PL1) and that one differed by one substitution at the 567 bp position (denoted as PL2). Both types of sequences had 676 bp and were deposited in GenBank under accession numbers KJ136024 and MW034586, respectively. The phylogenetic tree (Figure 3) inferred using maximum likelihood showed that the PL1 and PL2 sequences obtained in our study for *P. brachypomus* samples were nested within the Amazon Basin clade. The tree was generated with COI sequences with MEGA 7.0 using the Maximum Likelihood method based on the Kimura 3-parameter model. Bootstraps were shown at each node, and the scale bar indicated estimated substitutions per site. The *C. macropomum* and *M. duriventre* COI sequences were used as the outgroup.

Morphometric characters

The morphometric data are presented in Tables 1 and 2. The *TL−W* relationship of the fish examined shows Figure 4. The body of *P. brachypomus* was compressiform with a terminal mouth. The snout was more blunt in larger individuals. The body profile of the specimens examined was nearly straight from the dorsal fin insertion to the adipose fin origin. The body
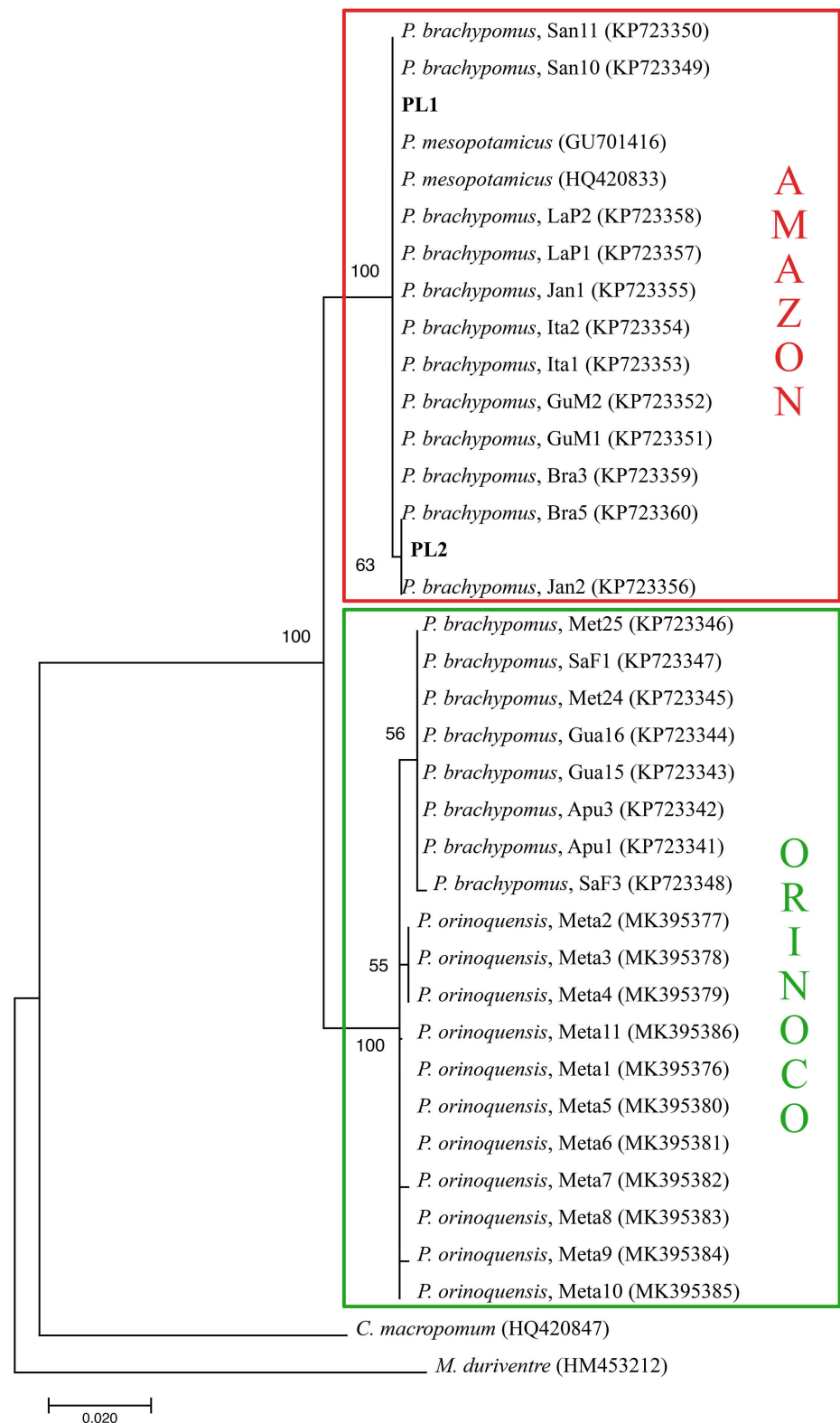
Figure 3. Phylogenetic tree of *Piaractus brachypomus* individuals from the Orinoco and Amazon basins. A phylogenetic tree was constructed with the Maximum Likelihood method (1000 bootstrap, Kimura 3-parameter model).

was covered with small cycloid scales, irregular in size, with a smooth caudal margin (Figure 5). They extended onto the base of caudal (4–5 rows), anal (6 rows), and adipose fins (7–9 rows) (Figures 1, 6). Scales were absent
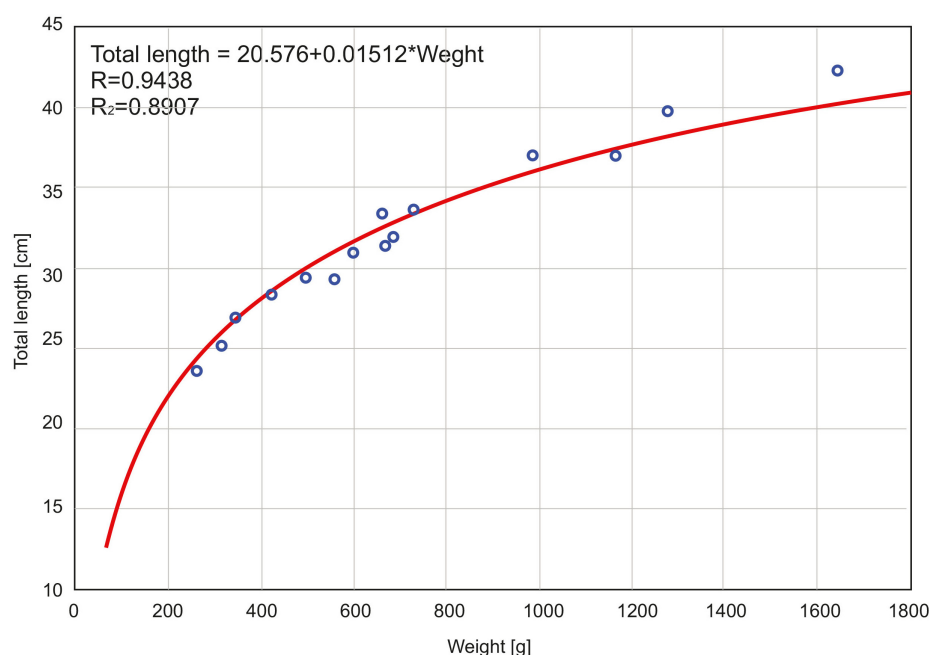
Figure 4. The total length *TL* – weight *W* relationship of the *Piaractus brachypomus* examined. Abbreviations: R – correlation coefficient; R^2 – coefficient of determination.
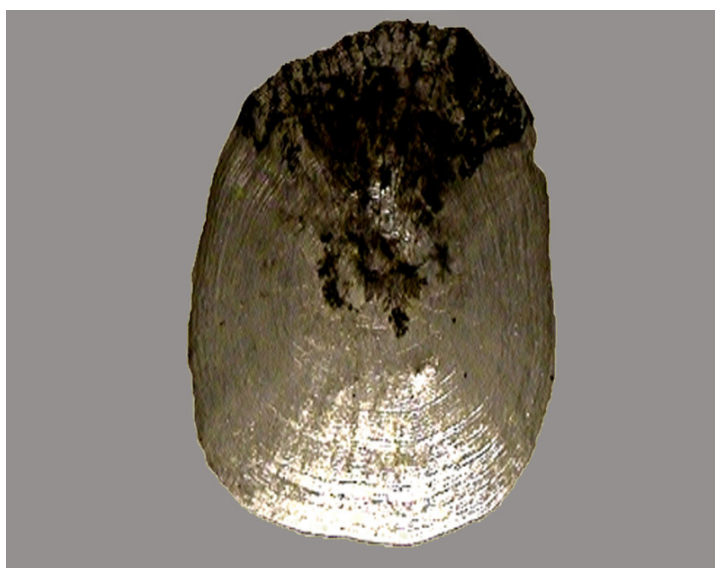

Figure 5. A cycloid scale collected from an individual *Piaractus brachypomus* aged 7+.

from the head and the supraoccipital crest. Body height was deepest at the vertical through the pelvic fin origin (approximately 46.5% of *SL* in both length classes). The caudal peduncle was slender (11.1% and 11.4% of *SL* in the two length classes) and relatively short (approximately 9.7% in both classes). The dorsal fin was situated in the middle of the back, and the adipose fin was closer to the caudal than to the dorsal fin; postdorsal distance was 34.6% and 36.3% vs. 19.0% and 22.5% of distance between dorsal and adipose fins in the two length classes, respectively. Predorsal distance was greater than postdorsal distance (the distance from the end of dorsal fin to the beginning of caudal fin) at 54.4% vs. 34.6% in the first length class and 52.8% vs. 36.3% in the second length class. Postdorsal distance
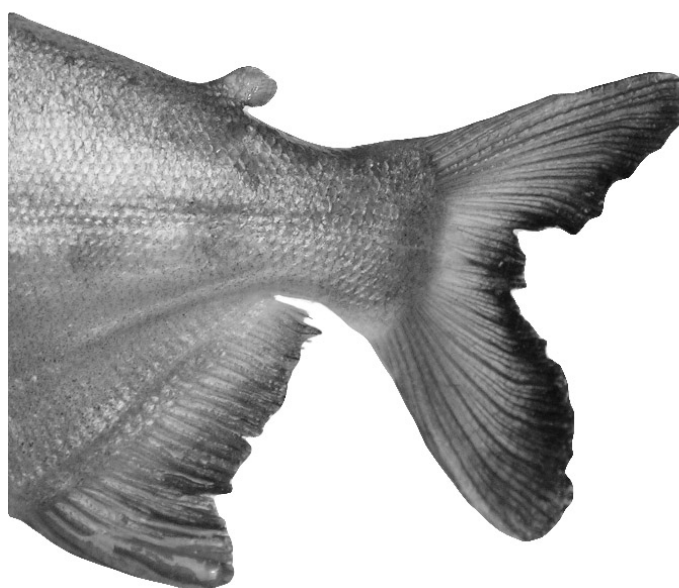
Figure 6. Shape of adipose fin in *Piaractus brachypomus*; the scales at the bases of the anal, adipose, and caudal fins are visible.

combined with dorsal fin base length were almost equal to predorsal distance. Pelvic fin origin was slightly anterior to the vertical through the dorsal fin origin. The anal fin base was slightly longer than the dorsal fin base (24.7% and 24.8% vs. 22.3% and 21.4% of *SL* in two length classes, respectively). The caudal fin was forked, and the lower lobe of caudal fin was longer than the upper one at 25.7% and 23.9% vs. 23.0% and 21.5% of *SL* in the two length classes, respectively. The adipose fin was rayless, with a rounded posterior dorsal edge and a convex fin base. The height of the scaled part of adipose fin constituted from 55.2% (second length class) to 64.8% (first length class) of the total fin height (Table 1).

The length of caudal peduncle, the height of dorsal and anal fins, and the length of pectoral fin were the most variable (from 17.7% to 28% of CV) as was the shape of the adipose fin (height of adipose fin, length of adipose fin base, and height of the scaled part of adipose fin). The following characters pertaining to the head were also highly variable (CV > 10%) in both length classes, but especially so in the first length class: preorbital length, postorbital length, head height and width, and particularly horizontal eye diameter (CV 18.9%). Student's t-test showed that the fish from the smaller length class had a statistically significant larger horizontal eye diameter and a smaller postorbital distance (relative to head length), and longer prepectoral length (relative to body length) (Tables 1, 2A).

Neurocranium morphometric data are presented in Table 2B. The cranium of *P. brachypomus* specimens studied was massive and high (Figure 7). Its depth at the highest point of the supraoccipital process was 54.7% and 53.8% expressed as percentages of cranial total length in the two length classes, respectively. The mesethmoid was relatively short, and had a pointed
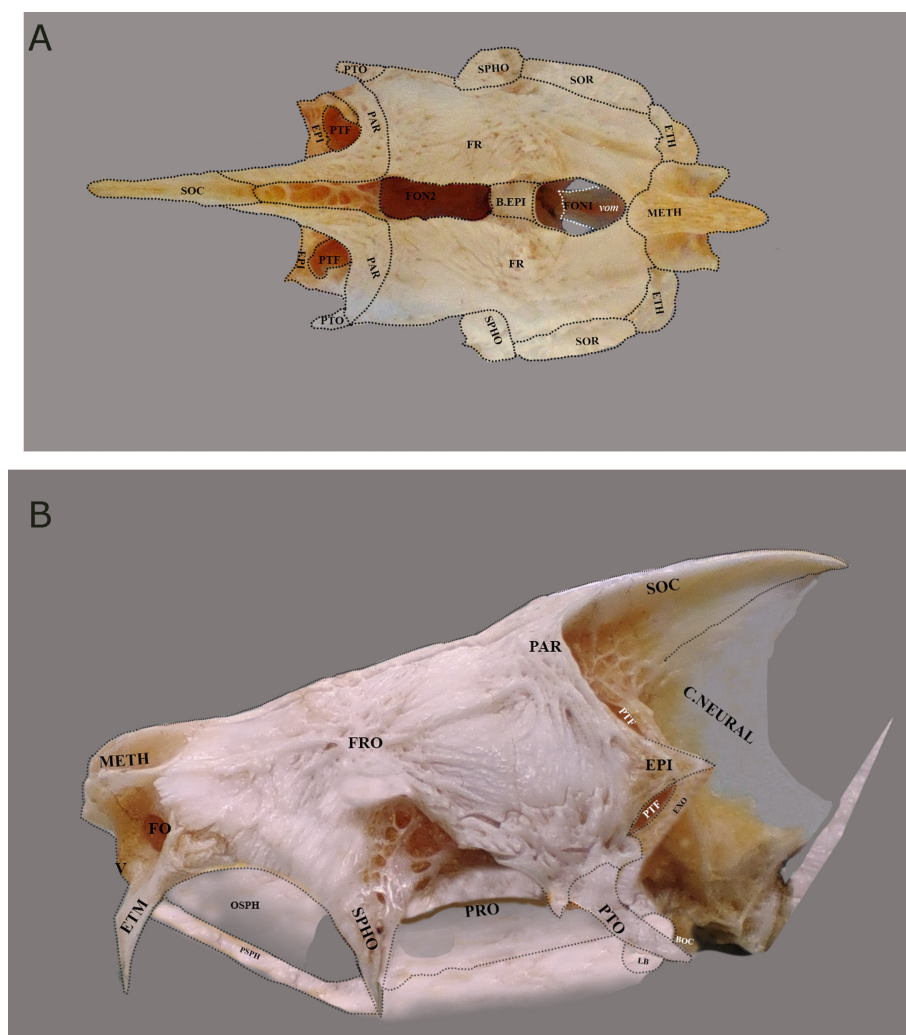
Figure 7. Neurocranium of *Piaractus brachypomus* collected from Polish water body; A dorsal view; B lateral view. Abbreviations: B.EPI – epiphyseal bar; BOC – basioccipitale; C.NEUR – neural complex; EPI – epioccipitale; ETH – ethmoidale laterale; EXO – exoccipitale; FO – fossa olfactive; FON$_1$, FON$_2$ – anterior (1) and posterior (2) part of frontal fontanel; FR – frontale; METH – mesethmoideum; PAR – parietale; PRO – prootic; PTF – post-temporal fossa; PTO – pteroticum; PSPH – parasphenoid; SOC – supraoccipitale crest; SOR – supraorbital; SPHO – sphenoticum; VOM – vomer; LB – lagenal bulla.

anterior projection that was relatively narrow. Its width was slightly wider in the first length class than in the second (16.7% and 15.4%, respectively). The mesethmoid contacted the frontal bones. The lateral wings of the ethmoideum were well developed, elongate, and ventrally directed. They were generally wider than the mesethmoid, and their widths were slightly wider in the second length class (41.3% vs. 42.4% of cranial length, respectively). The dorsal cranium roof was almost as wide anteriorly as it was posteriorly, with the widest part in the central section, while it was narrower anteriorly and posteriorly. The distance from anterior part of the vomer to basioccipitale was 55.7% and 53.9% of the cranial total length, while the broadest width of the cranium (i.e., the width of sphenotic bone) was 53.9% and 53.0% of the two length classes, respectively. Narrow, oval nasal bones were located along the lateral margins of the mesethmoid. The orbitosphenoid was high. The frontal bones (forming the majority of the

neurocranium roof) were sculptured radially. They were separated by the fontanel, which reached the parietal bones and also separated the halves of the left and right parietals. The parietals dorsal surface was also sculptured with many grooves, arranged laterally. The frontals did not contact each other anteriorly to the epiphyseal bar, which crossed the medial cranial fontanel transversely. The epiphyseal bar was slender and approximately cylindrical in cross section, without a laminar projection on the posterior margin. The width of the epiphyseal bar ranged from 7.5% to 7.8% of total cranium length, in the two length classes, respectively. The anterior margin of the frontal fontanel was formed by the posterior margin of the mesethmoid, while the distal edge of the fontanel was formed by the supraoccipital. The length of fontanel constituted up to approximately 38% of cranial length. The anterior part of fontanel was bullet-shape and was shorter than the posterior part (approximately 14% vs. 17.7% in both length classes, respectively). The posterior part of the fontanel was almost rectangular, with narrowing in the central part. The fontanel parameters did not change with fish growth, except for the width of the posterior part, which was insignificantly wider in larger fish.

The supraoccipital crest was highly developed, with a slightly convex dorsal profile. The crest was longer in larger fish at 40.1% vs. 33.6% of total cranium length in smaller fish, which was a statistically significant difference. The pterotic had two-pointed processes directed downward, and the first one was extended posteriorly as a long spine. The supraorbital was horizontally oriented. The parasphenoid was bent at an angle that ranged from 130 to 140°. The epioccipital had a lateral arm that extended toward the posterior margins of the parietal and pterotic, and separated the posttemporal fossa into the dorsal and ventral portions. The basioccipital formed the ventral surface of the saccular capsule. The exoccipital contained a large lagenar capsule (Figure 7A, B).

The relative characters of the neurocranium were not highly variable. Only the CV of the bottom length of neurocranium exceeded 10% in both length classes, while the CV of characters pertaining to the fontanel (except total length) exceeded 10% in the first length class (Table 2B). Of all the neurocranium measurements, only the relative length of the supraoccipital crest increased statistically significantly with fish growth, while relative sphenotic height, epioccipital width, and fontanel parameters decreased with growth; however, they were not statistically significant. The width of the posterior part of the fontanel increased slightly. Statistically significant relationships were noted among fontanel and supraoccipital crest lengths, epioccipital width, and lateral ethmoid height (Supplementary material Table S1).

The dentition formula is illustrated in Figure 8. The dentition of *P. brachypomus* was heterodont. The teeth were firmly ankylosed to the jaws. The premaxilla dentition included two series of rounded molariform
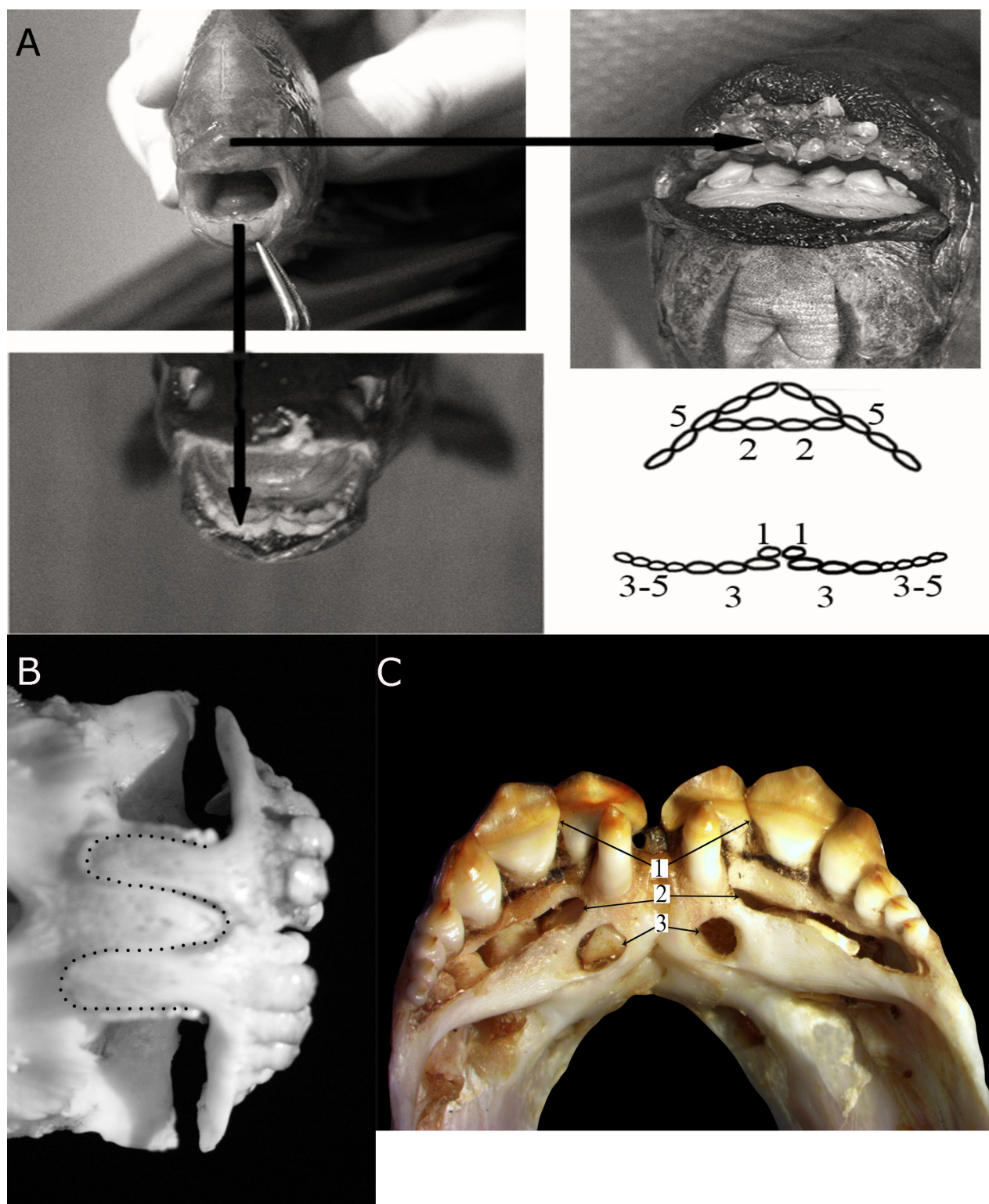
Figure 8. Dentition of *Piaractus brachypomus:* A – upper and lower jaws and the dentition pattern (premaxillary and dental bones); B – the junction of the premaxilla and the mesethmoideum; C – lower jaw structure: 1 – functional teeth with buttressed interlocking mechanism between symphyseal and adjacent (second) tooth; 2 and 3 – replacement tooth inside the bony crypts, present only on one side of jaw.

incisors: five teeth in the labial row (decreasing gradually in size backwards) and two in the lingual row. The outer series of teeth (3–3) were separated from inner the series (4) by a triangular hiatus (Figure 8A). The upper jaw had no visible interlocking mechanism. The premaxillary bones were heavily
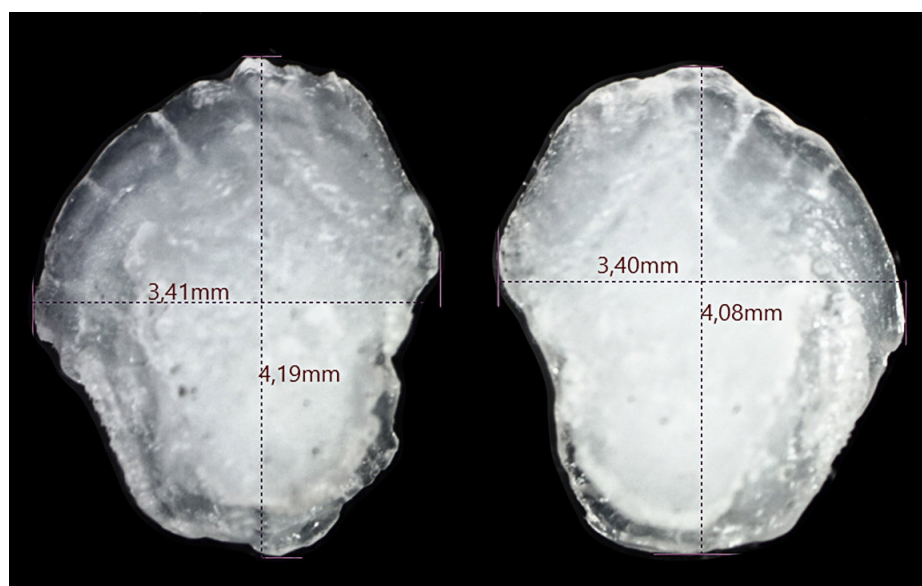
Figure 9. Shape of lapilli from individual of *Piaractus brachypomus* 31.3 cm TL.

attached to the mesethmoideum and were lacking interdigitations at the symphysis. The mesethmoid process almost completely separated the premaxillae. The ascending premaxillary process was elongate and reached the end of the nasal bone and was of similar width from the base to the rounded distal tip (Figure 8B). The specimens examined were edentulous on the upper anterior profile of the maxillary bone. One row of dentary teeth carried three large, strong teeth with triangular-shaped cusps with one longer conical inner symphyseal tooth and three to five small triangular-shaped teeth, decreasing markedly in size backwards. The lateral cusps clasped together. The replacement teeth were placed underneath the functional tooth row intraosseously within a "crypt" or bony chamber within the jaws only on one side of jaw (unilaterally) (Figure 8C). We noted two formulas in which the position of the smaller teeth was always asymmetrical: 3–3–3–4 and 4–3–3–5. Each formula was found in 50% of the specimens, and it was not correlated with head length. The lapillus shape of *P. brachypomus* is demonstrated in Figure 9. The length ranged from 3.40 mm to 3.41 mm, while the width ranged from 4.08 mm to 4.19 mm (left and right lapilli, respectively).

Meristic characters

The results of meristic studies are presented in Table 3. In a sample of pirapitinga examined from both natural waters and aquarium culture, three to four unbranched and 14–17 branched rays were noted in the dorsal fin, two unbranched and 22–26 branched rays were noted in the anal fin (three unbranched rays were noted only in two specimens). The pelvic fin had one unbranched ray and five to seven branched rays, whereas the pectoral fin had one unbranched and 13–18 branched rays. The ventral keel was composed of ventral-keel spines ranging from 48 to 66

Table 3. Meristic counts of the *Piaractus brachypomus* individuals (N = 16).

Character	Range	Mean ± SD	CV
Unbranched rays in dorsal fin *Du*	3–4	3.3 ± 0.5	14.8
Branched rays in dorsal fin *Db*	14–17	15.3 ± 0.9	5.8
Unbranched rays in anal fin *Au*	2–3	2.0 ± 0.3	13.9
Branched rays in anal fin *Ab*	22–26	24 ± 1.1	4.7
Unbranched rays in pelvic fin *Vu*	1	1 ± 0.0	0.0
Branched rays in pelvic fin *Vb**	5–7	6.5 ± 0.7	10.4
Unbranched rays in pectoral fin *Pu*	1	1 ± 0.0	0.0
Branched rays in pectoral fin *Pb*	13–18	15.1 ± 1.8	11.8
Ventral-keel spines K_1 (*P-V*)	27–44	33.8 ± 4.4	13.0
Ventral-keel spines K_2(*V-A*)	16–29	21.5 ± 4.3	20.1
Ventral-keel spines total *K*	48–66	55.3 ± 5.4	9.8
Scale rows above lateral line *l.l$_a$*	30–34	32.4 ± 1.7	5.2
Scales in lateral line *l.l*	90–115	103.6 ± 5.7	5.5
Scale rows below lateral line *l.l$_b$*	25–33	28.4 ± 2.9	10.1
Scale rows on adipose fin *ad.$_{sr}$*	7–9	8.5	9.8
Scale rows on anal fin *A$_{sr}$*	6	6.0	0.0
Scale rows on caudal fin *C$_{sr}$*	4–5	4.7	8.6
Gill rakers on first arch in first row *GR$_1$*	28–33	30.8 ± 1.9	6.1
Gill rakers on first arch in second row GR$_2$	12–16	14.3 ± 1.1	7.5
Vertebral count *vt**	36–37	36.3 ± 0.5	1.4

* statistically correlated with SL; p-level < 0.05
Abbreviations: M – mean; SD – standard deviation; CV – coefficient of variation.

(K_1 to the pelvics origin – from 27 to 44, and from the pelvics to the anal fin origin K_2 from 16 to 29). The lateral line was complete, and extended straight along its length and ended posteriorly between the middle caudal-fin rays. The perforated scale count was from 90 to 110, with an average of 103.6. The mean numbers of scale rows between the dorsal fin origin and the lateral line and between the lateral line and the pelvic fin insertion were 32.4 (range: 30–34) and 28.4 (25–33), respectively. The gill raker count on the first branchial arch in first row ranged from 28 to 33, while in the second row it ranged from 12 to 16. The vertebral count ranged from 36 to 37.

The coefficients of correlation calculated for the meristic characters and standard length *SL* were low, of low statistical significance, and ranged from −0.10 (lateral line scale count) to 0.42 (number of branched rays in the pelvic fin) and 0.48 (number of branched rays in the anal fin). Only the number of branched rays in the pelvic fin was significantly negatively correlated with standard length *SL* (−0.56), while the vertebral count – positively (0.97) (Table S2). The most variable (CV > 10%) characters were as follows: unbranched ray counts in dorsal and anal fins, branched ray counts in pelvic and pectoral fins, number of scale rows below the lateral line, and ventral-keel spine counts (Table 3).

Parasitological study

Neither external nor internal parasites were found in the individuals examined in this study.

Discussion

Pirapitinga is a tropical species and, despite its flexibility with regard to water temperature, the only areas where it could survive in Europe (without the possibility of reproducing) are probably heated post-cooling waters of power plants or in the southern regions of the continent. Therefore, it is considered a non-native species in Europe (Ribeiro et al. 2008; Więcaszek et al. 2016). However, its dietary plasticity, large body size, longevity, and capacity to achieve large abundances and wide distributions are characteristics that, according to invasive species theory, could have contributed to the successful establishment of *P. brachypomus* in Oceanian and Asian countries. In these countries this species finds conditions comparable to those in lowland South American rivers where migration and spawning occur in main river channel and floodplain habitats (Moyle and Marchetti 2006; Correa et al. 2014). The successful establishment in these countries, as well as in the U.S. (e.g., in South Florida), means that *P. brachypomus* has the status of an invasive species in these regions (http://www.evergladescisma.org/species/subinfo/?sub=18676).

In Poland, to date, nearly 30 records of this species (or specimens referred to as this species) have been noted since the first sighting in 2002. Specimens caught in water bodies were most likely released by aquarists (a part of a plastic plant was noted in the stomach of one specimen). However, only the samples from our study were investigated using molecular and morphometric characters. The samples of serrasalmid fish collected in Poland were confirmed as *P. brachypomus* based on COI sequencing. In addition to the molecular study, comprehensive morphometric procedures and comparisons of morphological and anatomical characters with those reported in Escobar et al. (2019) also indicated that all the individuals examined were the species *P. brachypomus*.

Individuals of *P. brachypomus* collected from the Orinoco and Amazon River basins have characteristic morphological features that enable their assignment to source populations (Escobar et al. 2015). Fish from the Amazon Basin were more robust, had shorter snouts, relatively larger eyes, and a larger distance separating the dorsal and adipose fins (Escobar et al. 2015). However, differences in morphological features among populations could be related to different habitat characteristics, such as temperature, turbidity, food availability, and water depth and flow (Turan et al. 2005). Rivas and Rujano (2013) found associations between environmental factors and morphological parameters in *P. brachypomus* individuals to differentiate this species in lentic and lotic environments in Venezuelan waters. Translocating fish specimens outside their native habitats also led to adaptations via phenotype modifications, which made species identification more difficult (Panicz and Keszka 2016).

Using the same COI marker as we did in the present work, Tiknaik et al. (2019) showed that the invasive fish described in the media as the red bellied

piranha *P. nattereri* was actually the omnivorous *P. brachypomus*. DNA barcoding is a powerful, widely-applied tool that also permits distinguishing new species from congeners as was evidenced for *P. orinoquensis* (Escobar et al. 2019). In addition molecular methods, Escobar et al. (2015) also analyzed morphological features to study the population structure of *P. brachypomus* in the Orinoco and Amazon basins, and this analysis revealed that each river basin was populated by distinct (average COI divergence of 3.5%), evolutionarily significant units (ESU). Sequences of pirapitinga from Poland (PL1, PL2) were phylogenetically nested within the Amazon Basin clade and were identical with haplotype San10 (KP723349) and Jan2 (KP723356), respectively (Figure 3). Our study also confirmed that DNA barcoding successfully supplemented morphological taxonomy to identify and cluster the *P. brachypomus* samples collected in Poland. Escobar et al. (2015) presented clear evidence that first generation hybrids between females from the Orinoco Basin and males from the Amazon Basin were most likely sterile. Nevertheless, since possible further hybridization or backcrossing remain unclear, further studies of *P. brachypomus* populations should consider using codominant markers (e.g., microsatellites) to avoid possible bias resulting from the maternal-line inheritance of mtDNA markers (Jorge et al. 2018).

In Poland, the U.S., and many Asian countries (recently including India, see: Tiknaik et al. 2019) numerous *Piaractus* spp. specimens and other closely related serrasalmids caught in open waters have been misidentified. The characteristic morphometric features of serrasalmids include a compressed body, a long dorsal fin with more than 16 rays (in this study the average number was 18.7), powerful dentition, and sharp ventral-keel spines, which result from a modification of the abdominal scales. Their number is variable with a range of six to nine in *Acnodon* to over 60 in *Piaractus* (Ortí et al. 2008).

The first sighting of serrasalmid representatives in Poland was widely publicized by the mass media as the occurrence of the traumatogenic *P. nattereri*. Indeed, the juvenile *P. brachypomus* has a distinct red chest and belly and displays dark grey to black spots and is often called the red-bellied pacu; thus it can easily be confused with the carnivorous red-bellied piranha, *P. nattereri*. This similarity is believed to be mimicry, which is an adaptation to avoid predation by other species (Lovshin 1995). Pirapitinga can be distinguished from piranhas primarily by dentition, which is a single row of serrated, incisor-like teeth in the piranha vs. two rows of strong molariform teeth on the premaxilla in the pacu. Pirapitinga is not aggressive, and its teeth are used for crushing fruits and seeds (Dahdul 2007). In its non-native distribution, however, *P. brachypomus* has earned the status of omnivore, when in the absence of its primary food of fruit and insects, it attacks other fishes causing ecological imbalance by preying on endemic fish species and their eggs and fry; attacks on humans have also been reported (Dau 2001).

Adult *P. brachypomus* lack a bright red chest and belly and resemble *P. orinoquensis*, *P. mesopotamicus*, *C. macropomum*, and their hybrids, but it can be separated by several meristic and morphological features. This species can be distinguished from *C. macropomum* by the adipose fin (rays are present in *C. macropomum* vs. absent in *P. brachypomus*, and the adipose fin is smaller), dentition (triangular gap between the premaxillary tooth rows in *P. brachypomus* vs. no gap in *C. macropomum*; teeth on the maxillary bone in *P. brachypomus* vs. no teeth on the maxillary bone in *C. macropomum*) (Ruiz-Carus and Davis 2003). According to Jégu (2003), in the genus *Piaractus* the maxillary bone presents one to three teeth on the upper anterior profile. In this study, however, the maxillae of the specimens examined were toothless.

All serrasalmids have interlocking teeth and share unusual unilateral tooth replacement, considered a synapomorphy for Serrasalmidae. All teeth on one side of the head are lost as a unit, then replaced simultaneously. The tooth cusp shape varies considerably among genera and species in pacus, but generally the upper jaw teeth have more rounded cusps whereas larger lower jaw teeth have triangular-shaped cusps. Kolmann et al. (2020, 2021), who mapped tooth replacement onto a molecular phylogeny, provided strong molecular support for recognizing the new subfamiliar rank of Colossomatinae. The new subfamily includes herbivorous pacus, common to lowland, black water, and white water habitats, with *Piaractus*, *Colossoma*, and *Mylossoma*.

Pirapitinga can be also distinguished from *C. macropomum* by opercle shape (short and not semilunate in *P. brachypomus* vs. semilunate in *C. macropomum*) and a more rounded head profile than *C. macropomum* (less elongated and pointed) (Lauzanna and Loubens 1985; Cagauan 2007). The next discriminative character between *P. brachypomus* and *C. macropomum* is the lapillus shape. Although relatively similar, lapillus shapes are species-specific, and the elliptical Fourier harmonic coefficients were sensitive in detecting these differences and efficiently quantified shape variability among species (Petry 2000). Another significant discriminative character among species could be the shape and size of the fontanel, which was documented in the genus *Clarias* (Więcaszek and Krzykawski 2010), but this needs further research in Colossomatinae.

Within the genus *Piaractus*, *P. brachypomus* differs from *P. mesopotamicus* and *P. orinoquensis* in the number of lateral line scales (84–100 in *P. brachypomus* vs. 107–128 and 72–89, respectively). Ruiz-Carus and Davis (2003) distinguished *P. brachypomus* from *P. mesopotamicus* by the limit of < 110 the lateral line scale count in the first species vs. > 110 in the second one. According to Jégu (2003), the lateral line of *P. brachypomus* is characterized by 88–98 scales, while Machado-Allison (1983) noted 70–89 scales (probably referring to *P. orinoquensis*). Hensel (2004) recorded 104 scales in one specimen studied, while Ross (2001) noted 97–102 scales in

the specimens studied. In this study, the lateral line scale count range was much wider, from 90 (one specimen) to 110 (two specimens), with a mode of 101 and an average of 103.57 that was higher than in Escobar et al. (2019). A significant discriminative character is also the scale rows above the lateral line (31–37 in *P. brachypomus* vs. 50–60 in *P. mesopotamicus* and 23–30 in *P. orinoquensis*), and scale rows below the lateral line (33–37 in *P. brachypomus* vs. 49–56 and 22–32, respectively) (Escobar et al. 2019). The results of the present study showed the number of scale rows were less numerous; both above and below the lateral line. The number of ventral-keel spines of *P. brachypomus* was similar to other studies (Jégu 2003; Machado-Allison 1983; Čaleta et al. 2011).The mean count of prepelvic and postpelvic ventral-keel spines in this study, however, was higher when compared to data given in Escobar et al. (2019), for both *P. brachypomus* and *P. orinoquensis*. The average count of rays in pectoral fin in this study was close to the average count reported in Escobar et al. (2019) for *P. brachypomus*, while it was lower in *P. orinoquensis*. In turn, the average number of rays in the anal fin was close in both species. The average count of rays in the pelvic fins in this study was lower in comparison to data from Escobar et al. (2019) for both species. The range of all fin rays count, however, was wider in the fish from the current study. The range of vertebral counts in the fish from this study was with the range in Escobar et al. (2019) for *P. orinoquensis,* but it is higher than for *P. brachypomus.* Similarly, the range of the tooth count on the dentary (the smaller ones) was larger than that reported in Escobar et al. (2019).

The current study showed that the morphometric characters of the *P. brachypomus* body and head were highly variable in both length classes. Escobar et al. (2019) presented the detailed morphometric characteristics of *P. brachypomus* from its native location (in the sample most specimens were to 30.0 cm *TL*), while Hensel (2004) and Čaleta et al. (2011) published information on the characteristics of some *P. brachypomus* morphometric characters from small, single individuals caught in European waters (< 25 cm *SL*), but these were not confirmed by molecular studies. Machado-Allison (1983) described a population from Venezuelan waters (Orinoco Basin) as *P. brachypomus*, which is currently recognized as *P. orinoquensis*. The values of the morphometric characters presented in this study were highly variable. In the current study the comparison of the results of morphometric characters in two length classes indicated that fish size can markedly affect the values of these characters. Discrepancies among the results could also result from differences in study areas, habitat types, food resources, and the number of fish examined. Morphometric characters do not seem to be appropriate tool to distinguish populations or closely related species in the *Piaractus* genus and are comparable only within suitably selected samples.

The structure of the *P. brachypomus* neurocranium has not yet been well documented, and to date only a few characters have been described (Escobar et al. 2019). In general, the neurocranial morphology of serrasalmid species can be influenced by a variety of factors: phylogenetic history, morphology of feeding apparatus, body shape, and sensory morphology. Variation in habitat and feeding behavior can require certain sensory modalities, such as vision and hearing, to play greater roles (Boyle and Herrel 2018). The *P. brachypomus* neurocranium is characterized by the great development of the supraoccipital spine. Machado-Allison (1983) proposed a correlation between the great development of the supraoccipital spine and the absence of predorsal scales and he considered the absence of predorsal scales as a synapomorphy of the Serrasalminae (= Serrasalmidae), and a long pterotic spine as a feature observed in members of the pacu clade, especially *C. macropomum* and *P. brachypomus*, what was confirmed in this study in the case of latter of these species (Figure 6B).

In the natural environment of *P. brachypomus*, the parasitic fauna is represented by 12 metazoan species, namely Monogenea (*Anacanthorus spathulatus* Kritsky, Thatcher & Kayton, 1979, *Mymarothecium viatorum* Boeger, Piasecki & Sobecka, 2002, *Notozothecium janauachensis* Belmont-Jégu, Domingues & Martins, 2004), Digenea (*Clinostomum marginatum* (Rudolphi, 1819) Braun, 1899, *Dadaytrema oxycephalum* (Diesing, 1850) Vaz, 1932), Nematoda (*Spectatus spectatus* Travassos, 1923, larvae of *Contracaecum* sp. Railliet & Henry, 1912), and Crustacea (*Argulus carteri* Cunnington, 1931, *Ergasilus* sp. von Nordmann, 1832) (Oliveira and Tavares-Dias 2016). Recently, two new myxobolid species were recorded (*Henneguya brachypomus* Capodifoglio, Adriano, Naldoni, Meira, da Silva & Maia, 2020 and *Myxobolus pirapitingae* Capodifoglio, Adriano, Naldoni, Meira, da Silva & Maia, 2020) (Capodifoglio et al. 2020). Thatcher (2000) described a new species of parasitic copepoda, *Lernaea pirapitingae* (= *Perulernaea pirapitingae*) (Thatcher, 2000), from *P. brachypomus* from the Meta River (Orinoco Basin), which is recognized currently as *P. orinoquensis*. The monogenic fluke *M. viatorum,* specific to this host, was described for the first time in a specimen of *P. brachypomus* collected in Polish waters, and this is the only report of a neotropical monogenoid in a neotropical fish caught in European waters (Boeger et al. 2002). The absence of other parasite species in this study could have resulted from the short residence time of these fish in open waters. Di Santo et al. (2018) showed that the ability of *P. brachypomus* to adapt to new environments depended on water temperature. A short period of stay in water with lower temperature may limit the colonization of this host by parasites (Combes 2001). Although in its natural environment pirapitinga is mainly herbivorous (Dau 2001), in our study the stomach of one specimen contained cyprinid scales. All the other specimens caught in Polish waters had empty gastrointestinal tracts. Trophic plasticity increases the probability this

species could be colonized by parasites from local waters that have complex development cycles since hosts ingest with food parasitic development stages or adults (Oliveira and Tavares-Dias 2016). Our parasitological study was the second such study performed on this species in Europe. With the exception of the description in Boeger et al. (2002), all specimens in our study were free of parasites. Nevertheless, alien fish species released into European waters should be monitored continually.

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Authors' contribution

Conceptualization: BW; Methodology: BW, RP, JD, AL, SK; Formal analysis and investigation: BW, RP, JD, AL, SK, AB; Writing – original draft preparation: BW, RP, JD; Writing – review and editing: BW, RP, AL; Funding acquisition: West Pomeranian University of Technology in Szczecin.

Ethics and permits

This article does not contain any studies with animals testing performed by any of the authors. Fish from the waters bodies were delivered dead to our laboratory, and fish from aquarium culture died in natural way (within 2012–2019). Moreover, the release of live pirapitinga into natural environment is forbidden in Poland.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Correlations among the morphometric features of the neurocranium, and the neurocranium features with standard length, $p < 0.05$ (N = 16).

Table S2. Correlations among the meristic features, and meristic features with the standard length, $p < 0.05$.

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