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Short Communication

Possible progenesis in *Neophasis anarrhichae* (Nicoll, 1909) Bray, 1987 in the White Sea

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ABSTRACT

Neophasis anarrhichae (Nicoll, 1909) Bray, 1987, unlike the majority of acanthocolpid digeneans, has an abbreviated two-host life cycle. The reproduction of rediae, development of cercariae, and their transformation into unencysted metacercariae occur within the only intermediate host, the whelk *Buccinum undatum*. Normally, the metacercariae develop into sexual adults (maritae) and egg production starts when the infected whelk is eaten by a wolffish *Anarhichas lupus*. In the White Sea, we have found three cases of infection of *B. undatum* by progenetic metacercariae of *N. anarrichae*. These metacercariae had a fully developed and functioning hermaphroditic reproductive system, and eggs were found in their uterus. Most eggs observed in the histological sections were abortive, but some contained embryos at early stages of development. The progenetic metacercariae were similar in their morphometric characteristics to the sexual adults from the wolffish, the main differences being the size of the ovary and eggs. In order to confirm progenesis, and thus a facultative one-host life cycle in *N. anarrichae*, we need to prove that the eggs from metacercariae are viable.

Neophasis anarrhichae (Nicoll, 1909) Bray, 1987 (Acanthocolpidae) is one of the typical intestinal parasites of the wolffish *Anarhichas lupus* Linnaeus, 1758 [1]. It is most abundant in North-Eastern European marine waters, with the highest prevalences recorded in the White and Barents Seas [1–3]. The majority of known acanthocolpid life cycles involve three hosts [1]. *Neophasis anarrichae* is an exception with a two-host life cycle which is considered to be derived [4,5]. The only intermediate host is a common whelk, *Buccinum undatum* Linnaeus, 1758. Rediae develop in the digestive gland and gonad of the whelk and produce cercariae. The cercariae lose their tail while still within the redia and become metacercariae which do not encyst [6,7]. The wolffish becomes infected by eating a whelk containing metacercariae, which then become sexual adults in the intestine and produce eggs. However, while studying endoparasites of *B. undatum* from the White Sea, we found three whelks with peculiar infestation of *N. anarrichae*: the metacercariae within rediae contained developing eggs within their uterus, and eggs were found laid into the brood chamber of rediae. This may agree with a recent definition of progenesis in trematodes [8]. Here we describe these progenetic metacercariae and compare them with sexual adults (maritae) from *A. lupus* and to normal non-progenetic metacercariae from the whelk.

The whelks *B. undatum* and wolffish *A. lupus* were collected in the White Sea, Kandalaksha Bay, near the Educational and Research Station “Belomorskaia” of Saint Petersburg State University (SPbU) during 2015–2018. They were dissected, tissues were examined in physiological solution (0.6% NaCl for whelks and 0.9% for wolffish), and parasites were picked. We studied cases of infections with (1) typical metacercariae of *N. anarrhichae* in *B. undatum* (Fig. 1b), (2) progenetic metacercariae in *B. undatum* (Fig. 1c), and (3) normal adults from the wolffish (Fig. 1a). All in all, we found three whelks containing progenetic metacercariae: specimen 1 in August 2015 (30 whelks examined), specimen 2 in April 2016 (21 whelks examined), and specimen 3 in July 2018 (88 whelks examined). During the whole period (2015–2018) we also found seven whelks with typical metacercariae of *N. anarrhichae*. The worms were flat-fixed in 70% ethanol and mounted in Mowiol® 4–88 (Sigma) either unstained or stained with borax carmine. For the histological study of progenetic metacercariae, pieces of whelk digestive gland were fixed in Zenker's solution, dehydrated in a series of

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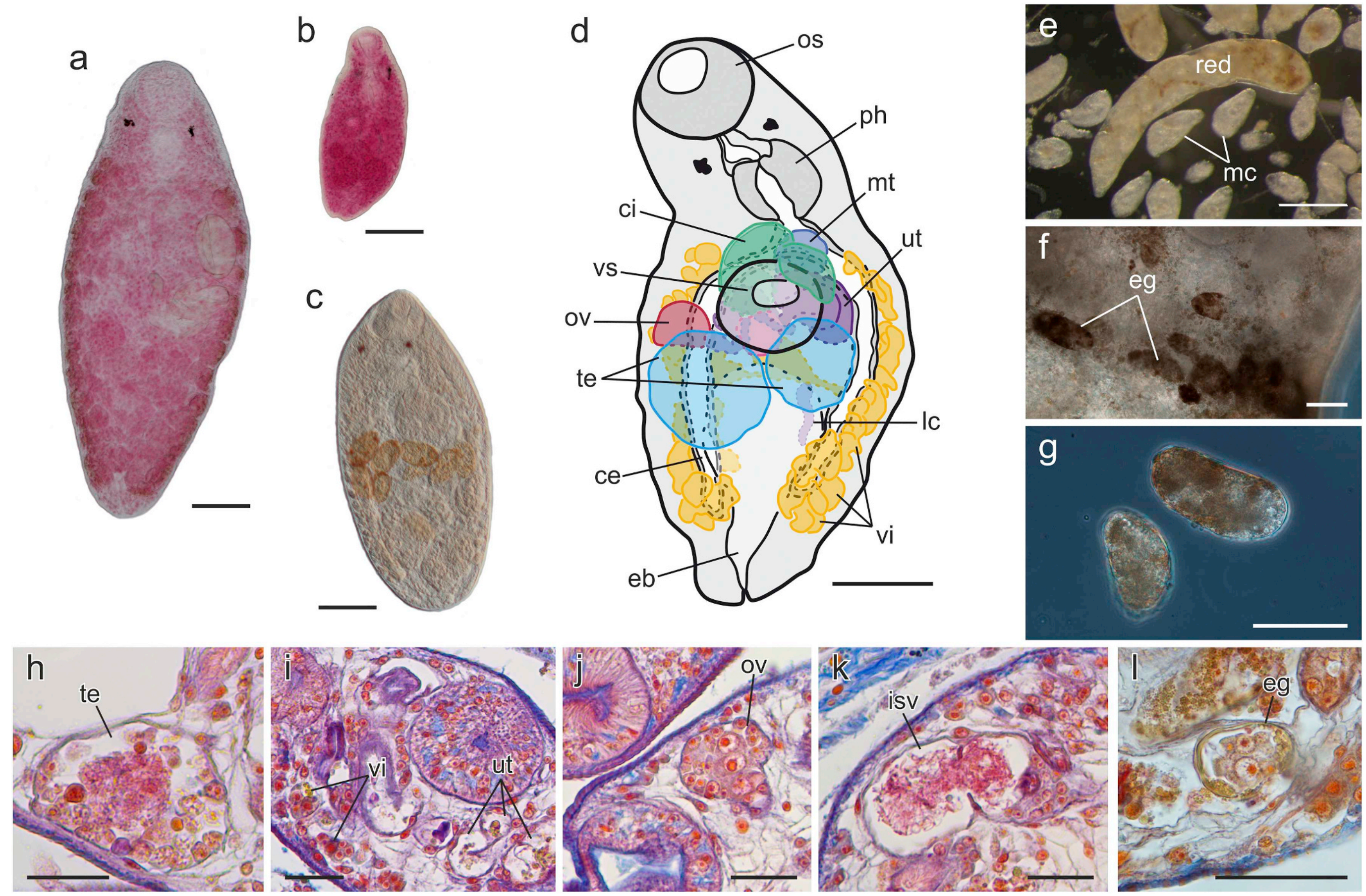

Fig. 1. a. Sexual adult from *A. lupus* (carmine stained). b. Metacercaria from *B. undatum* (carmine stained). c. Progenetic metacercaria from *B. undatum* (unstained). a-c are shown at the same scale. d. Diagram of progenetic metacercarial organization: cecum (*ce*), cirrus (*ci*), excretory bladder (*eb*), Laurer's canal (*lc*), metraterm (*mt*), oral sucker (*os*), ovary (*ov*), pharynx (*ph*), testes (*te*), uterus (*ut*), vitelline follicles (*vi*), and ventral sucker (*vs*). e. Rediae (*red*) and progenetic metacercariae (*mc*), specimen 2. f. Eggs (*eg*) in brood chamber of redia (DIC). g. Eggs from progenetic metacercariae (DIC). h–l. Histological sections of progenetic metacercariae (Mallory's trichrome stain): testis (*te*), ovary (*ov*), vitelline follicles (*vi*), uterus (*ut*), internal seminal vesicle (*isv*), and eggs (*eg*) are visible.
Scale bars on a–d – 100 μm, on e – 500 μm, on f–l – 50 μm.

graded alcohols, and embedded in Histomix™. Sections were cut at 5 μm and stained with Mallory's trichrome stain. Photographs of live rediae containing progenetic metacercariae were taken using differential interference contrast microscopy (DIC) of temporary mounts.

Standard statistical methods were used to analyze the morphometric data obtained from the whole mounts [9]. P-values < 0.05 were considered significant. To visualize the complex differences and similarities between adults, normal and progenetic metacercariae with respect to the size of their somatic and reproductive parts, we calculated matrices of the Bray-Curtis dissimilarity between specimens and then used these for two different non-metrical multidimensional scalings (nMDSs). To reduce the effects of specimen deformation during fixation, we used areas of the body and organs (approximated by ellipses) rather than linear sizes. All calculations were performed using the R statistic environment [17].

In specimen 1, we found rediae of different ages containing cercariae at different stages of development, cercariae transforming into metacercariae, formed metacercariae, and metacercariae containing developing eggs (Fig. 1c). In each individual redia only one of these groups was dominant. The most numerous were normal metacercariae, slightly exceeding the progenetic ones. All listed stages of development were present in specimen 2, although metacercariae containing eggs and cercariae were rare. However, almost all full-grown rediae contained eggs which had obviously been laid by progenetic metacercariae (Fig. 1e–g). In specimen 3, singular progenetic metacercariae were found. The study of histological sections showed that the progenetic metacercariae had a fully developed hermaphroditic reproductive system (Fig. 1d, h–k). Their seminal vesicle was filled with sperm (Fig. 1k) and they had eggs in the uterus. Most eggs observed in histological sections were abortive, but some contained early stages of embryonic development (Fig. 1l).

Average measurements of *N. anarrichae* progenetic metacercariae with normal fully formed metacercariae from *B. undatum* and ovigerous adults from *A. lupus* are shown in the Table 1, and supplemented with the measurements of adults by Bray and Gibson [1] and measurements of adults and normal metacercariae by Chubrik [6]. According to the nMDS plot based on the sizes of somatic parts, the 95% confidence intervals (CIs) for the centroids of two groups (adults and progenetic metacercariae) overlapped with each other and the CI for the third centroid (normal metacercariae) was separated from the other two (Fig. 2a). The differences were due to all measured somatic body parts (body, both suckers, pharynx). On the nMDS plot based on the sizes of reproductive organs, the CIs for all three groups were separate, the progenetic metacercariae were situated between the adults and normal

Table 1
Measurements of *N. anarrichae* adults, normal and progenetic metacercariae.

	Our data			Bray and Gibson, 1991	Chubrik, 1966	
	Adults from *A. lupus*	Metacercariae from *B. undatum*	Progenetic metacercariae from *B. undatum*	Adults from *A. lupus*	Adults from *A. lupus*	Metacercariae from *B. undatum*
Body length (μm)	504–735 (665.6 ± 38.2)	337–528 (430.5 ± 34.1)	432–571 (501.4 ± 46.6)	383–615 (489)	550–660	210–380
Body width (μm)	265–431 (341.9 ± 25.8)	144–218 (180.6 ± 12.6)	255–368 (301.4 ± 34.8)	101–335 (220)	190–260	90–170
Ventral sucker diameter 1 (μm)	79–118 (89.6 ± 6.7)	56–78 (69.1 ± 4.6)	77–90 (84.2 ± 3.6)	51–105 (70)	80–100	40–60
Ventral sucker diameter 2 (μm)	85–146 (108.7 ± 8.9)	50–76 (65.5 ± 5.4)	88–118 (100.6 ± 8.3)	61–98 (76)	–	–
Oral sucker diameter 1 (μm)	83–122 (98.5 ± 6.1)	57–81 (67.9 ± 4.7)	68–101 (86.3 ± 10.3)	48–94 (69)	80–90	50–60
Oral sucker diameter 2 (μm)	97–121 (105.9 ± 4.2)	59–85 (74.5 ± 5.3)	62–109 (87.7 ± 12.9)	47–95 (67)	–	–
Pharynx diameter 1 (μm)	66–96 (78.6 ± 4.7)	51–68 (6.7 ± 3.7)	53–85 (69 ± 8.4)	47–80 (62)	40–50	20–50
Pharynx diameter 2 (μm)	55–95 (70.1 ± 6.0)	40–61 (52.6 ± 4.2)	55–75 (66.3 ± 6.1)	46–70 (55)	–	–
Ovary diameter 1 (μm)	68–90 (78.0 ± 7.7)	29–44 (35.5 ± 3.3)	57–81 (65.8 ± 7.6)	45–88 (64)	60–70	20–50
Ovary diameter 2 (μm)	58–68 (64.4 ± 4.1)	24–42 (32.8 ± 3.6)	46–84 (57.3 ± 12.2)	38–68 (49)	–	–
Anterior testis diameter 1 (μm)	107–139 (107 ± 10.3)	50–88 (70.7 ± 7.2)	82–110 (99 ± 7.6)	48–139 (82)	50–70[a]	20–30[a]
Anterior testis diameter 2 (μm)	69–91 (81.0 ± 7.1)	40–74 (56.3 ± 6.7)	53–77 (70.1 ± 7.3)	37–80 (66)		
Posterior testis diameter 1 (μm)	98–145 (119.7 ± 16.2)	52–85 (67 ± 6.1)	85–111 (95.5 ± 8.4)	54–92 (69)		
Posterior testis diameter 2 (μm)	66–98 (78.6 ± 12.6)	42–67 (55.1 ± 5.7)	61–85 (78.8 ± 8.6)	35–85 (66)		
Egg length (μm)	72–121 (103.4 ± 30)	–	67–85 (72.9 ± 5.6)	76–85	–	–
Egg number	0–8 (2.1 ± 1.1)	0	0–8 (1.5 ± 2)	1–5	3–4	0

[a] The author gives one interval for both testes.

metacercariae (Fig. 2b). According to the plot, the separation was mainly due to the ovary size. The size of testes didn't contribute substantially. The eggs observed in the uterus of progenetic metacercariae were significantly smaller than those from adults (Student *t*-test, $p < .0001$, $n1 = 30$, $n2 = 7$).

The first description of *N. anarrhichae* in the intermediate host was given by M. Lebour along with the data on its prevalence at the eastern coast of Great Britain [10]. Also, extensive studies on endoparasites of the common whelk were held in the Danish waters and at the Barents Sea [6,7]. These authors had much larger numbers of whelks infected by *N. anarrhichae* than we did, however they did not report progenetic metacercariae. Thus, we for the first time describe the progenesis in *N. anarrhichae*, and in the whole fam. Acanthocolpidae as well.

Present data show that the progenetic metacercariae are almost identical in their morphometric characteristics to the adults from the wolffish. This does not fit the formal definition of progenesis by Gould [11], but follows the meaning of this term in the parasitological literature – “the attainment of both sexual and somatic development in the non-definitive host” [8]. The main differences between the sexual adults and progenetic metacercariae of *N. anarrhichae* relate to the size of the ovary and eggs and to egg production (most eggs appear to be abortive in progenetic metacercariae). This may be due to heterochrony in reproductive system development, as it appears that the female reproductive organs start to function later than the male organs. Such protandrous development in digeneans had been previously described at least for some representatives of the Microphallidae [12].

Miracidial development is unknown for *N. anarrichae* or any other species of *Neophasis*, and we had no chance to perform any experimental infections. Thus, we are unable to determine whether the eggs produced by metacercariae are capable of completing their development. If they are invasive for another whelk, this may lead to elucidation of one more facultative one-host life cycle, which are quite rare in Digenea [13].

Factors which may favor the evolution towards progenesis and life cycle truncation were summarized by F. Lefebvre and R. Poulin [8] and R. Poulin and T. Cribb [13]. Among the proposed hypotheses [13], *the developmental time hypothesis* seems to be the most suitable in case of *N. anarrhichae.* It states that the cause of progenetic development is the time spent by the parasite inside its intermediate host regardless of transmission probability [13,14]. The other hypotheses are less relevant in our case: *the environmental instability hypothesis* – due to the conditions in the White Sea subtidal; *the rare or missing host hypothesis* – as wolffish is abundant in the White Sea all over the year and remains active at least during all summer months; *the latitudinal gradient hypothesis* – as no cases of progenesis for *N. anarrhichae* were registered in the Barents Sea; and *the cannibalism hypothesis* – as the egg formation in *N. anarrhichae* is more likely to be completed in the external environment rather than within the uterus of a sexual adult (considering the short uterus and much yolk in the egg). However, complex experiments must be conducted to prove the effect of any of the factors in each host-parasite system [14–16]. The absence of a metacercarial cyst is also thought to favor the acquisition of progenesis [8] and that is the case of *N. anarrhichae.*

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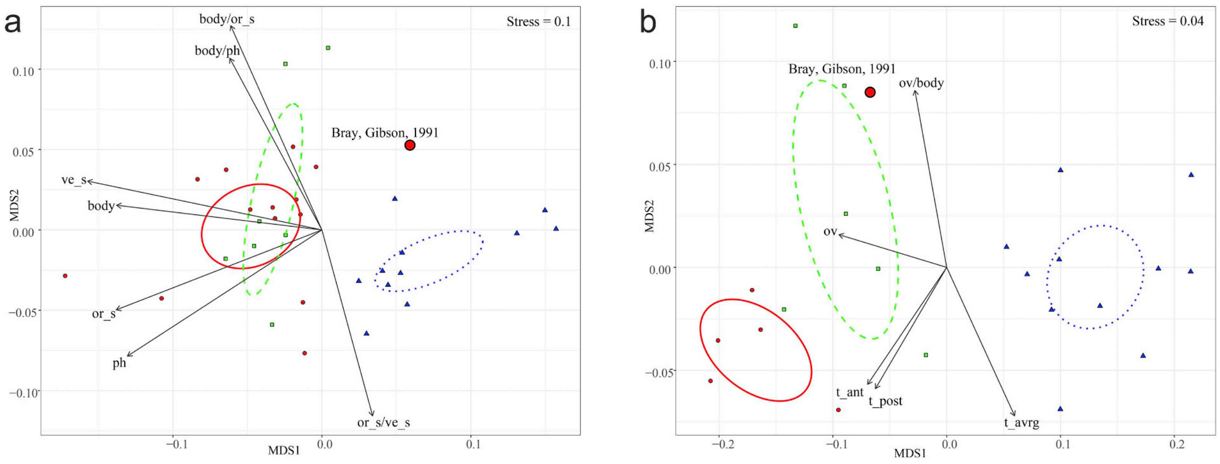

Fig. 2. NMDS ordinations based on sizes of somatic (a) and reproductive (b) parts of adults (*circles*, *solid line*), normal metacercariae (*triangles*, *dotted line*) and progenetic metacercariae (*squares*, *dashed line*) of *N. anarrhichae*. The ellipses represent 95% confidence intervals of the group centroids. Original variables significantly correlated with the NMDS axes are presented as arrows to demonstrate their contribution to the observed patterns. The large circle represents the data of Bray and Gibson (1991) (mean values). Original variables: *body* — body area, *ph* — pharynx area, *ve_s* — ventral sucker area, *or_s* — oral sucker area, *body/ph* — body area-pharynx area ratio, *body/or_s* — body area/oral sucker area ratio, *body/ve_s* — body area/ventral sucker area ratio, *ov* — ovary area, *test_a* — anterior testis area, *test_p* — posterior testis area, *test/ov* — testis area (averaged)/ovary area ratio, *ov/body* — ovary area/body area ratio).

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