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Early Researchers Involved with Branchiobdellidans (Annelida: Clitellata) on Japanese Crayfish, and a Reassessment of the Taxonomic Status of *Branchiobdella digitata* Pierantoni, 1906

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Abstract: During a visit to the Musée des Confluences in Lyon, France, specimens of the branchiobdellidan *Cirrodrilus cirratus* were discovered on four endemic Japanese crayfish, *Cambaroides japonicus*, that had been donated to the museum in 1889. This discovery was placed in context with a review of the early international researchers involved with studying Japanese branchiobdellidans. Concomitantly, investigations on *Cirrodrilus inukaii*, including morphological variations, led to a reassessment of the taxonomic status of *Branchiobdella digitata* Pierantoni, 1906. This resulted in *B. digitata* being transferred to the genus *Cirrodrilus* as *C. digitatus* (Pierantoni, 1906) comb. n. and *C. inukaii* (Yamaguchi, 1934) recognised as a junior synonym of *C. digitatus* (new synonymy). These studies have demonstrated the importance of good preservation practices and particularly the need in future decapod collections so as to conserve the present diversity of their delicate ectosymbionts.

Key words: Ectosymbiosis; Japanese branchiobdellidans; *Cirrodrilus* spp., taxonomy; *Cirrodrilus digitatus* comb. n.; *Cirrodrilus inukaii*, a synonym of *C. digitatus*; *Cambaroides japonicus,* host.

Introduction

Interest in natural history started to increase in the 18th century. This led to the formation of many public and private museums and coincided with the introduction of Linnaean taxonomy. Although it has been recognised for many years that museums and private collections catalogued a wide variety of animals, their records rarely include information on accompanying symbionts or more particularly parasites. However, the presence on crayfish of ectosymbiotic branchiobdellidan annelids (Hall 1914, Subchev & Gelder 2010, Subchev et al. 2017) has led to new species descriptions, correcting misnamed specimens, expansions of range distribution and other information in Europe (Subchev 2011, 2014), North America (Holt & Opell 1993) and East Asia (Timm 1999, Ohtaka et al. 2020).

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Following the discovery of branchiobdellidans on Japanese crayfish in the Conservation Centre and Collection Studies (CCEC) of the Musée des Confluences (previously called the Natural History Museum of Lyon, MHNL) in Lyon, France, by one of us (J-FP), the authors realised that the early history of branchiobdellidans and the researchers involved with Japanese material, was more extensive than the information reported in Pierantoni (1905, 1906, 1912) and was worthy of reporting. This research coincided with an extensive accumulation of observations on *Cirrodrilus inukaii* (Yamaguchi, 1934) made by SRG and AO, which suggested it was time to re-examine the questionable taxonomic status of *Branchiobdella digitata* Pierantoni, 1906.

Historical review of early researchers of branchiobdellidans associated with Japan

The oldest existing specimens of branchiobdellidans in Japan come from *Cambaroides japonicus* (De Haan, 1841) collected by an unknown person in 1872 from Lake Akan, Hokkaido (Nakata et al. 2014). Although it is not known where the specimens were kept after collection in 1872, they were incorporated into the Dr. Saburo Hatta Collection at Hokkaido University in Sapporo for safe keeping after he was appointed Museum Director in 1908 (Inukai 1936). [Note: Japanese names are given in the European sequence with given name first followed by family name.] On examination by Nakata et al. (2014), these crayfish were found to be carrying *Cirrodrilus cirratus* Pierantoni, 1905, *C. inukaii* and *Cirrodrilus megalodentatus* (Yamaguchi, 1934).

During the late 19th century, many young, later to be eminent zoologists, studied in Europe, North America and Japan, with students and mentors becoming part of a communications network of researchers in natural history. In 1875, Charles O. Whitman from Maine, USA, studied for a few months at the University of Naples, Italy, before moving to the University of Leipzig, Germany, where he received his Ph. D. in zoology in 1878. He spent the next two years, from 1879 to 1881, at the Imperial University in Tokyo, Japan, as the second professor of zoology (Mayr 2019). While in Leipzig, presumably after returning from Japan, Whitman (1882) collected local "*Astacus fluviatilis*" (= *A. astacus*), which carried branchiobdellidans, for the purpose of comparing them with three species found on "*A. japonicus*" (= *C. japonicus*). The few morphological details Whitman gave were insufficient to make a species identification but he promised a full description in a future paper. A search of Whitman's publications listed in Morse (1912) did not find such a paper. Following Whitman's time at the University of Naples, Professor Francesco S. Monticelli joined the Institution in 1888 and accepted the Chair of Zoology and the directorship of the Zoological Museum in 1900. It is not clear when but during a search of preserved freshwater decapods for ectosymbiotic Gondwanan temnocephalidans, Monticelli also recovered branchiobdellidans from the gills and exposed surfaces of Japanese "*Astacus*". According to Oka (1907), these Japanese "*Astacus*" were collected in Sapporo, Hokkaido, by unknown persons and sent to the French National Museum of Natural History, Paris, France, by the Minister, Mr. Harmand, at the French Embassy in Tokyo. Monticelli sent the specimens on to Umberto Pierantoni, who on graduation in Natural History studies at the University of Naples in 1899, was appointed by Monticelli as his assistant in the Institute for Zoology. Despite the specimens being poorly preserved and distorted, Pierantoni (1905) described the first branchiobdellidan from East Asia and named the new species, *Cirrodrilus cirratus* Pierantoni, 1905. Subsequently, Yamaguchi (1932) provided a detailed description of the species using well preserved material.

Oka (1907) reported that Pierantoni asked Prof. Isao Ijima (also spelt Iijima) of the Imperial University in Tokyo for new Japanese branchiobdellidan material, which resulted in Ijima sending new specimens collected in Sapporo directly to Pierantoni in Naples. Ijima had been a student under Whitman during his tenure at the Imperial University and almost certainly was familiar with branchiobdellidans. Further examination of the Paris Museum specimens and those newly arrived from Ijima, led Pierantoni to describe *Branchiobdella digitata* Pierantoni, 1906, and *Stephanodrilus sapporensis* Pierantoni, 1906; the generic name of the latter was relegated to a junior synonym of *Cirrodrilus sapporensis* (Pierantoni, 1906) following the Principle of Priority in a review of the genus by Holt (1967). Pierantoni (1906) recognised the problems of using poorly preserved specimens and stated that he hoped to obtain additional well-preserved material from Japan. The crayfish referred to in Pierantoni (1906) were reported to have been collected from "Jesso" (= Yeso or Yezo) Island, Japan, which caused confusion to some researchers, until it was realised that the island's name was changed in 1869 to the modern, Hokkaido Island. Unlike the description of *C. cirratus*, illustrations of internal organs in both *B. digitata* and *C. sapporensis* could only have been made if a few specimens were reasonably preserved (Pierantoni 1906). Even so this did not

preclude some anomalous features being described, e.g., a second set of ovaries in segment 8 of *C. sapporensis* (Pierantoni 1906: Tav. 5, Figs 12 and 13). Yamaguchi (1934: 204) stated: "Other characters of the genital organs [of *C. sapporensis*] do not differ from species of *Stephanodrilus* treated in this paper"; hence, he did not find a second pair of ovaries.

Professor Asajiro Oka had become interested in Japanese branchiobdellidans in the early 1900s, which led to a short paper (Oka 1907) in which he included the three Japanese species described in Pierantoni (1905, 1906). Kawamura (1918) was the last Japanese publication on branchiobdellidans in this period and he reported "*St. sapporensis, Pterodrilus* sp. = *St. cirratus* and *Branchiobdella* sp. = *St. kawamurai*" according to a correction made in Yamaguchi (1934). Yamaguchi (1934) briefly reviewed the history of branchiobdellidans and reported on his studies of worms from Manchuria, the Korean Peninsula, and particularly Japan. While post Yamaguchi (1934) research on East Asia branchiobdellidans is beyond the scope of this review, references to them can be found in Ohtaka et al. (2020).

Background of the taxonomic status of *Branchiobdella digitata*

The description of *B. digitata* was based on an unknown number of specimens and Pierantoni (1906) did not designate any types or say where the material was deposited. From the historical review above, it would be logical for the specimens to have been kept in either the Naples or Paris museums but recent searches in those institutions' collections have not found the material and it is presumed lost. Pierantoni placed the species in the *Branchiobdella* because he observed only a single pair of testes and those were in segment 5 in a sperm sac (Fig. 2E); however, no species in this genus has been reported in Japan, contrary to Subchev (2008: 236). Timm (1991: 328) believed the second pair of testes in segment 6 were overlooked due to the poor state of preservation. The dorsal jaw (Fig. 2C and D) shows an anterior curving large median tooth and 12 small teeth on the convexed anterior margin of the jaw base (Fig. 2C). Yamaguchi (1934: 192), at the end of his description of *Cirrodrilus inukaii* (Yamaguchi, 1934), stated: "The original figures of *Branchiobdella digitata* given by Pierantoni (1906) generally accord with the present species in external features and structure of dental plates. But the former possesses one pair of testes and male funnel (Pierantoni 1906, 1912), while the latter is provided with two pairs of those organs [testes]." Subsequently, Timm (1991: 328) considered, "*B. digitata* a *Cirrodrilus* sp., perhaps close to *C. inukaii* (Yamaguchi, 1934)", which caused him to suggest it be regarded as a "questionable species".

Materials and Methods

Four *Cambaroides japonicus* were discovered in a 70% alcohol-filled jar, MHNL.44000786, at the Conservation Centre and Collection Studies (CCEC) of the Musée des Confluences (MHNL) in Lyon, France (Parpet et al. 2019). The sex and body length (measured from rostrum point to the telson margin) of each crayfish were recorded. Then the crayfish and bottom debris from the jar were examined for branchiobdellidans and cocoons (Table 1). Five branchiobdellidans were selected randomly, cleaned of extraneous material, dehydrated in successively increasing ethanol solutions, cleared with methyl salicylate and mounted individually on slides in Canada balsam for examination on a Leitz compound microscope using bright-field, dark-field and phase contrast illumination. Slides of the mounted specimens were assigned registration numbers (MHNL 44000786.1 to 44000786.5) with unmounted specimens being stored in 70% ethanol (MHNL 44000786.6).

Pierantoni (1906: 1-2, Tav. 5, Figs 1-5) provided the description and figures for *B. digitata*. Those for *C. inukaii* come from Yamaguchi (1934: 192-196, Figs 3A-G, 4A-B). For brevity and ease of comparison, all of the figures by the two authors have been redrawn and appear in our Fig. 2A-E for *B. digitata* and Fig. 3A-F for *C. inukaii* and are cited as such in the text. Neither author placed scale bars on their figures, but they did specify the magnification, at which the image was drawn, and this factor has been used to calculate the scale bars shown.

Over 100 specimens of *C. inukaii* have been examined by AO and SRG, including some individuals, which were accidentally preserved in overly dilute preservative (about 45% ethanol) (Gelder, unpub. obs.).

Results

Each of the four crayfish specimens from the Lyon Museum were assigned an uppercase letter (Table 1) and can be identified by their total length and sex. Eleven branchiobdellidans and 38 cocoons were recovered, and their locations described. The worms showed varying degrees of bodily distortion, appeared yellow to orange, and some internal regions were opaque (Fig. 1) indicating they had been partially desiccated in the past.

Table 1. Branchiobdellidans and cocoons discovered on four *Cambaroides japonicus* and in the bottom debris of jar MHNL.44000786 dated 1889.

Host no.	Sex	Length (mm)*	Site	Worm numbers	Cocoon numbers
A	Male	65.1	Chelae	0	2 (0)
B	Male	31.3	Chelae	3	8 (4)
C	Female	64.1	Chelae	3	8 (3)
			PII	0	10 (4)
			PIII	0	10 (5)
D	Female	49.3	--	0	0 (0)
Bottom of the jar				5	0 (0)
Total				11	38 (16)

*Length was measured from rostrum point to the telson margin. PII and PIII represent the second and third pairs of pereopods or walking legs, with cocoon numbers in parentheses to indicate empty cases.

Fig. 1. The five *Cirrodrilus cirratus* in 70% alcohol. (Leica MZ16 binocular dissecting microscope with oblique lighting, scale bar = 0.5 mm. Photographed by Jean-François Parpet).

The jaws were clearly recognisable in three specimens (MHML 44000786.2, 44000786.4 and 44000786.5) along with peristomial details in the latter two. It was also possible to count the dorsal segmental appendages on a few segments and this enabled them to be identified as *C. cirratus*; however, no internal anatomy could be discerned in any of the specimens. Slide 44000786.1 contained a cocoon that had been removed from a seta. The only other possible species with the same peristomium and jaw characters is *Cirrodrilus uchidai* Yamaguchi, 1934, but the number of dorsal segmental appendages did not favour this species. Although no collection site was recorded for the crayfish, *C. cirratus* is only found on Hokkaido Island, Japan (Ohtaka et al. 2020).

Reassessment of the taxonomic status of *Branchiobdella digitata*

The jaws are the most resistant structure to distortion in the worm's body, and while great reliance is placed on their morphology in a species description and for identification, they are subject to intraspecific variation and their appearance will vary depending upon the aspect observed. Pierantoni (1906) only illustrated the dorsal jaw (Fig. 2 C and D) and this showed a large median tooth curving anteriorly with 12 small teeth on a convexed margin (Fig. 2C) plus a small area of additional base posteriorly. Subsequently, Yamaguchi (1934: 192) described *C. inukaii* with an anteriorly curving dorsal median tooth and smaller teeth along a straight anterior margin (Fig. 3D). The appearance of the small teeth along the jaw margin looks strange until they are seen in lateral view (Fig. 3E). Although Figs 2C and D showed 12 small teeth, Yamaguchi only reported 6; however, in the latter, the large median tooth obscures the number of small teeth below it. Examinations of over 100 specimens of *C. inukaii* by AO and SRG have found the number of small teeth on the dorsal jaw varies between 8 and 12 or four to six pairs. The convex anterior margin (Figs 2C and D) and the straight margin (Fig. 3D) could be a diagnostic character for separating the two species; however, having been able to examine large numbers of *C. inukaii*, a few specimens appear to have a convex margin and this was due to subtle differences in the orientation. A large median tooth is present in most *Cirrodrilus* species but only three, *C. inukaii*, *Cirrodrilus aomorensis* (Yamaguchi, 1934) and *Cirrodrilus iwakiensis* Ohtaka & Gelder, 2015, have a curved dorsal tooth. In the latter two species, the tooth curves posteriorly and they are not found on Hokkaido Island.

The body shape of branchiobdellidans can vary greatly and is dependent on the worm's reaction to preservation conditions during the short time before death occurs. In that stress period, the branchiobdellidan will usually either extend or contract; Pierantoni shows an extended spindle or terete reaction (Figs 2A and B), while Yamaguchi's specimen (Fig. 3A) has contracted. This is not of taxonomic importance as Yamaguchi (1943: 192) explains: "In resting or contracted state it is club-shaped, and more or

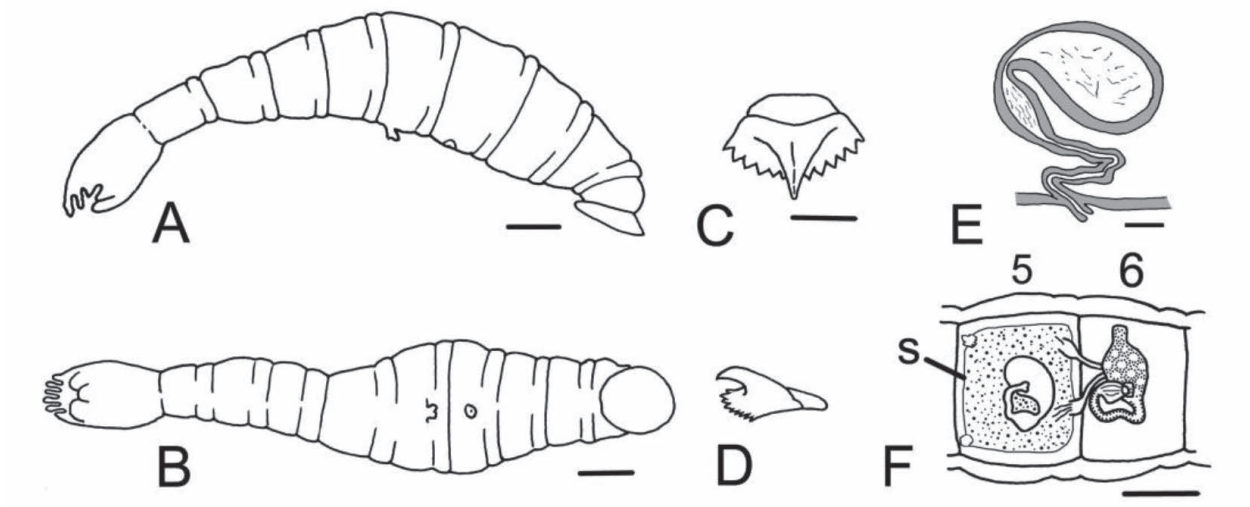

Fig. 2. *Branchiobdella digitata*: A, lateral view; B, ventral view; C, dorsal jaw ventral view, median tooth pointing anteriorly; D, dorsal jaw oblique lateral view, median tooth pointing anteriorly; E, diagrammatic vertical section of the spermatheca with spermatozoa in the bulb and duct dilation; F, ventral view of segments 5 and 6, showing sperm sac (s) with developing spermatozoa and spermatheca in 5, and male organs in 6. Scale bars: A and B = 0.2 mm; C & D = 10μm; E = 30μm; F = 0.1mm. A-F redrawn from illustrations accompanying the original description in Pierantoni (1906), with scale bars calculated from the magnifications given on the original drawings: A and B, x50; C & D, x900; E, x250; F, x60.

less flattened in the posterior region; while in fully extended state it is rather cylindrical throughout the whole length with gradually narrowed anterior portion." An extended body condition with contracted, rounded peristomial appendages was also observed in specimens exposed to an overly dilute preservative (45% ethanol) (Gelder, unpub. obs.).

The arrangement of peristomial tentacles is the next most important character after jaw morphology in the identification of *Cirrodrilus* species. Unfortunately, these structures are sensitive to even slight localised distortions during preservation and mounting. The dorsal peristomial lip according to Pierantoni (1906) (Fig. 2B) has six short tentacles but the outer pair of tentacles appear slightly shorter and in Fig. 2A they appear more like one of the two lateral lobes. Such an arrangement agrees with that shown in *C. inukaii* (Fig. 3C). The lower lateral lobe in Fig. 2A is retracted behind the rounded ventral lip, while Fig. 2B shows one rounded pair of lateral lobes ahead of the central rounded ventral lip. This discrepancy could indicate that two different specimens were observed but in both drawings the ventral lip has contracted to eliminate the median emargination and small lateral ventral lip lobe visible in Fig. 3B and C. A few extended body specimens of *C. inukaii* described above in dilute preservative also had peristomial appendages similar in appearance to those in Fig. 2B (Gelder unpub. obs.). Further, the oldest specimens known from Japan (Nakata et al. 2014: Fig. 2B) contained *C. inukaii* showing four dorsal club-shaped tentacles and similar contracted lateral and ventral lobes. A comparable distortion of the lateral lobes and ventral lip was reported by Pierantoni (1912: Tav. 5, number 1) in *Branchiobdella minuta* Pierantoni, 1912, but this appearance was shown to be a preservation artifact following an examination of a well-preserved head of *B. minuta* found in the Hamburg Museum, Germany (Gelder 1987: 18).

Pierantoni (1906) described spermatozoa in a sperm sac in segment 5 (Fig. 2E: s) but not in segment 6, hence the specimen was assigned to the genus *Branchiobdella*. As spermatozoan development occurs free in the coelomic cavity in branchiobdellidans, it is not clear what Pierantoni identified as a sperm sac. Timm (1991: 328) believed the second pair of testes had been overlooked due to poor preservation. While this could be true, the quality of preservation seen in the detail in segment 5 would not support a preservation problem, and no explanation can be offered to account for the missing testes.

Drawings of the spermatheca (Fig. 2E and F) show an ental pyriform bulb connected to a dilated portion of the spermathecal duct before it terminates in a spermathecal pore surrounded by an external papilla. Spermatozoa are clearly represented in the bulb and dilation (Fig. 2F) indicating that the organ was well preserved. This form of a spermatheca is unique to *Cirrodrilus* as described by Yamaguchi (1934: 194) and Gelder (1987: 20, 22). The shape of the glandular atrium in a species of *Cirrodrilus*

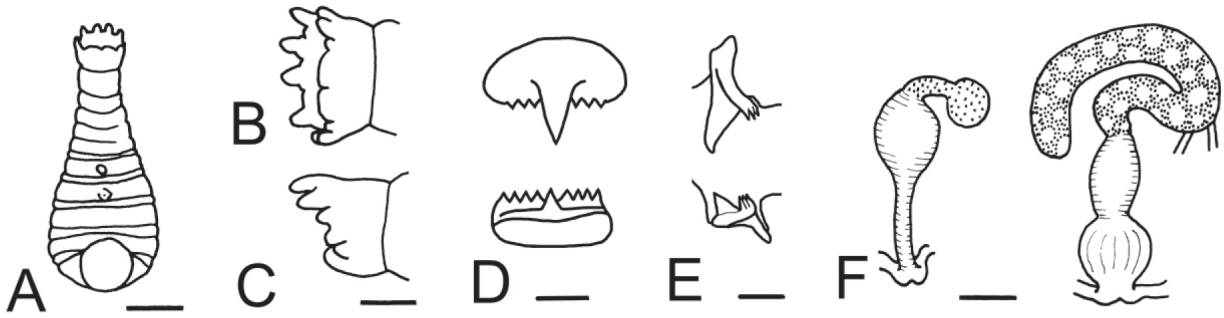

Fig. 3. *Cirrodrilus digitatus* (Pierantoni, 1906): A, ventral view; B, ventral view of peristomium; C, lateral view of peristomium; D, dorsal jaw over ventral, small teeth anterior; E, lateral view of dorsal jaw over ventral, mouth to the right; lateral views of spermatheca in segments 5 (horizontal lines = spermathecal duct; dotted = spermathecal bulb) and male organs in segment 6 (dotted = glandular atrium; horizontal lines = ejaculatory duct; vertical lines = bursa), respectively. Scale bars: A = 0.2 mm; B and C = 80 μm; D = 15 μm; E = 15 μm; F = 50 μm. A-F redrawn and modified from illustrations accompanying the original description of *C. inukaii* in Yamaguchi (1934), with scale bars calculated from the magnifications given for the original drawings.

varies from curved cylindrical (Fig. 3E) to pyriform (Gelder 1987: 20, 22), with an intermediate form shown in Fig. 2E. This variability is due largely to the reproductive condition of an individual rather than a preservation method. Even though the sperm ducts originate differently (Figs 2E and 3F), they both enter the glandular atrium separately and at approximately the same position.

Discussion

The historical review shows the importance of personal academic connections and their influence on the early research of branchiobdellidans in Japan. The discovery of *C. japonicus* carrying branchiobdellidans is an example of specimens being translocated by a non-scientist wishing to enrich the natural history collection in his home city museum. While the specimens have not added to the historical Japanese record, they have demonstrated the importance of searching old records and collections for forgotten material.

The apparent loss of the material used to describe *B. digitata* has place great emphasis on the importance of the published details and relating them to more recent studies. It is not known how many specimens Pierantoni examined but the differences noted in the number of dorsal lip tentacles, lateral lobes and the lengths of the contracted ventral lips (Fig. 2A and B) would support at least two specimens. The different aspects reported of the dorsal jaw were observed at a magnification of x900, which would have required an oil immersion objective with a very small working distance. It is possible a single specimen mounted in a clearing fluid could be examined in a ventral position (Fig. 2C) and then rolled to show an oblique view (Fig. 2D) but the chances of causing damage would be very high. The absence of information on the ventral jaw is strange and inexplicable. The drawings of the spermatheca indicate that two of more specimens were examined as the vertical section through the organ (Fig. 2E) contrasts with the horizontal orientation shown from a ventral position (Fig. 2F).

In summary, the differences in body shape, dorsal jaw morphology and peristomial appendages have been shown to be within intraspecific variations observed in *C. inukaii*. The spermatheca and male organs are also consistent with this species. However, Pierantoni's reported absence of testes in segment 6 cannot be explained and, given the morphological details reported in segment 5, poor preservation does not fully account for the omission. Therefore, following the International Code of Zoological Nomenclature (ICZN 1999) *Branchiobdella digitata* Pierantoni, 1906 is an available name (Article 11.1) and recognised as a valid species by the present study. However, the species was placed in the wrong genus and is therefore transferred to *Cirrodrilus* Pierantoni, 1905 as justified by Holt (1967: 3) as *Cirrodrilus digitatus* (Pierantoni, 1906) comb. n. Furthermore, *Cirrodrilus inukaii* (Yamaguchi, 1934) now becomes a junior synonym of *Cirrodrilus digitatus* (Pierantoni, 1906) (new synonymy) based on the assessment presented.

Crayfish and other acceptable crustacean hosts for branchiobdellidans in museums provide the only information on past species distributions and possible unknown species of ectosymbionts that may now be extinct. Therefore, ensuring these crustaceans remain immersed in preserving alcohol and the bottom debris in their containers is not discarded, is

the only chance for uncovering the recent history of branchiobdellidans. Given the world-wide decrease in biodiversity, the value of future crustacean collections with their attendant symbionts, would be greatly enhanced by placing these potential hosts directly into, and maintaining them in the appropriate concentration of alcohol, and discontinuing the damaging practice of an initial immersion in formalin or freezing.

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