

## On the type specimens of the common freshwater shrimp *Palaemon paucidens* De Haan, 1844 collected by Von Siebold and deposited in Naturalis Biodiversity Center

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### Abstract

Recent molecular analyses revealed *Palaemon paucidens* De Haan, 1844 to be species complex, since three genetically distinct types (designated by types A, B and C) were detected. A mitochondrial 16S rDNA sequence of the lectotype specimen collected by P. F. von Siebold nearly two centuries ago in Japan and held in Naturalis Biodiversity Center (formerly Rijksmuseum van Natuurlijke Historie), Leiden, The Netherlands, was successfully determined to be haplotype JA1 of type A. JA1 is the most predominant haplotype mainly observed from central to southern part of Japan and would be the most accessible type to Von Siebold whose activity range was limited around Nagasaki.

**Key words:** *Palaemon paucidens*; type specimen; type A; mitochondrial DNA

Dr. Philipp Franz von Siebold (Fig. 1) stayed in Nagasaki, Japan from 1823 to 1830. He was a great contributor to the study of Japanese fauna and flora. The *Fauna Japonica*, a series of monographs on the zoology in Japan, is mainly based on collections made by Von Siebold. The common freshwater shrimp *Palaemon paucidens* (Fig. 2) is one of the species collected by Von Siebold and described in the *Fauna Japonica* by De Haan (1833-1850). *P. paucidens* is widely distributed in various inland waters of northeast Asia and has been considered to be the sole native species of the genus inhabiting freshwater environments in Japan. However, two genetically distinct types (designated as types A and B) in *P. paucidens* were first detected by allozyme analysis (Chow and



Fig. 1. Portrait of Philipp Franz von Siebold drawn by Keiga Kawahara, 1820s.

Fujio 1985; Chow et al. 1988; Fidhiany et al. 1988), which were lately supported by 18S rDNA sequence analysis (Chow et al. 2018a). Not only large genetic divergence between these two types was further confirmed by mitochondrial (mtDNA) 16S rDNA sequence analysis, but another distinct type (designated as type C) was also detected (Chow et al. 2018b). Mean nucleotide sequence divergence (K2P distance) in 16S rDNA between these three types was 5.5 to 6.8 %, which is well within the distance range between species of the genus *Palaemon* (Murphy and Austin 2003; Carvalho et al. 2014). Not substantial but slightly divergent clades (A-I and A-II) were also observed within type A. In addition, type B was further subdivided into clades B-I and B-II, between which substantial genetic divergence (K2P = 3.7 %) was observed (Chow et al. 2018b). Individuals belonging to clade B-I were observed to be distributed in northern Japan (north of Miyagi Prefecture to Japan Sea side), while those belonging to clade B-II were observed south of Miyagi Prefecture (Chow et al. 2018b). Collecting locations of these types and clades are represented in Fig. 3 (modified from Chow et al. 2018b). Recently, Katogi et al. (2019) alleged the clade B-I to be a new species and described it as *Palaemon septemtrionalis*. Thus, *P. paucidens* appears to be a species complex, and therefore the affiliation of the lectotype specimen collected by Von Siebold to the aforementioned types or clades needs to be determined.

Other than the information ‘collected in Japan’, the three type specimens (one lectotype and two paralectotypes) of *P. paucidens* (RMNH.CRUS. D.1027) (Fig. 4) held in Naturalis Biodiversity



Fig. 2. *Palaemon paucidens*. a: type A collected in Lake Tsukui, Kanagawa, Japan, b: type B collected in Tozawa River, Shizuoka, Japan.

Center are not accompanied with detailed collecting information. One individual (designated by 1027-1) carried eggs, and we expected the eggs may be used for DNA analysis unless they were fixed in formalin. Crude DNA was extracted from the eggs using a DNA extraction kit (Genomic Prep Cell and Tissue DNA Isolation Kit, Amersham Bioscience). Since the first attempt to amplify a partial sequence of mitochondrial 16S rDNA using universal primer pair (16Sar-L and 16Sbr-H) (Palumbi et al. 1991) failed, we designed an internal primer pair for nested PCR. Nucleotide sequences of the primers for nested PCR were 5'-AAAGTCTAACCTGCCCACTG-3' (16SPpNF) and 5'-TTCAACATCGAGGTCGCAAC-3' (16SPpNR) based on conserved regions between different haplotypes of *P. paucidens*. Amplification of a fragment was observed in the 2nd PCR. Direct nucleotide sequence analysis was

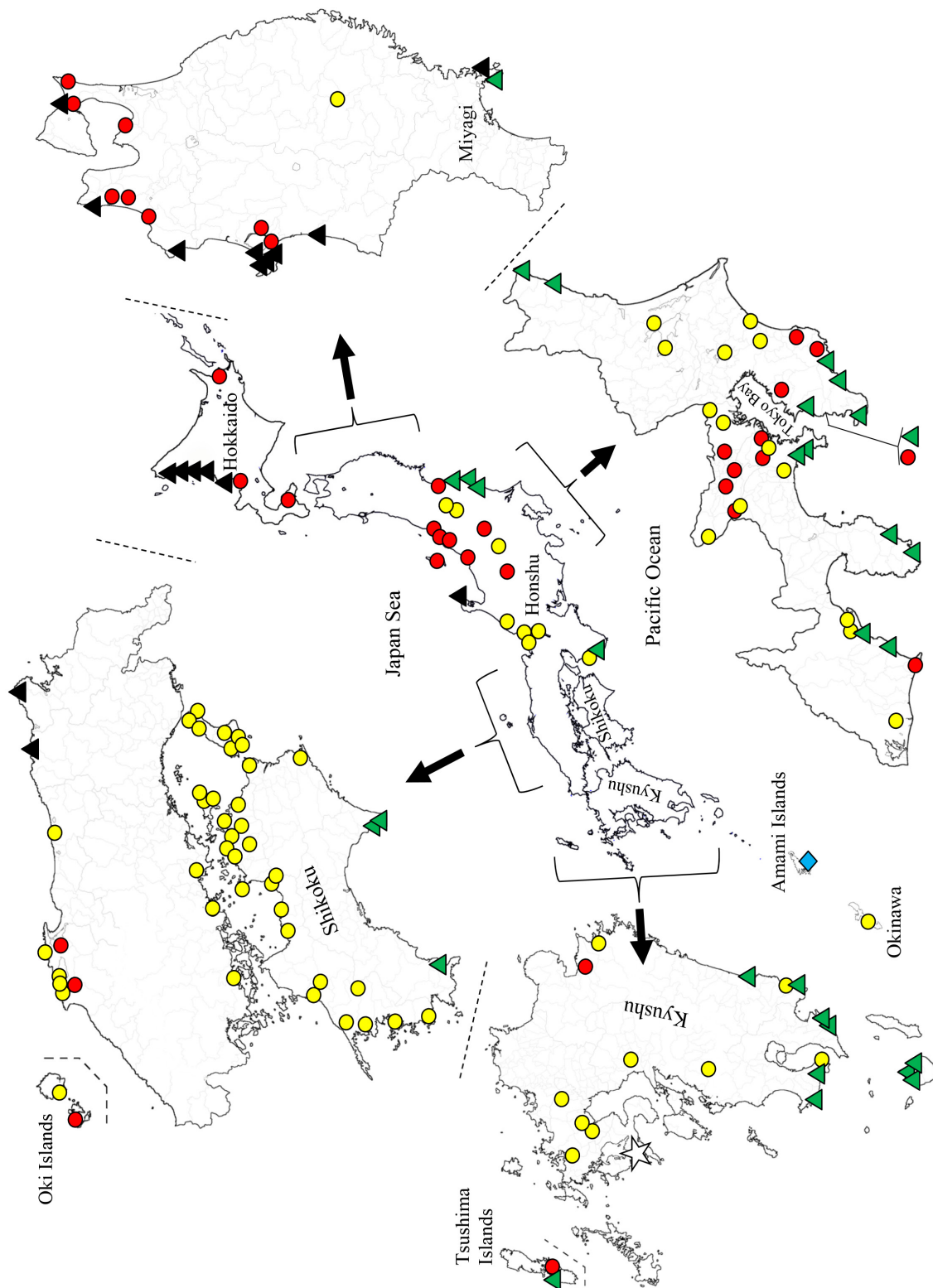


Fig. 3. Map showing collection localities of type A (circle), type B (triangle), and type C (blue diamond) of *Palaemon paucidens* (data obtained from Chow et al. 2018b). Types A and B are respectively subdivided into two clades (I and II); yellow circle (A-I), red circle (A-II), black triangle (B-I), and green triangle (B-II). Star indicates Nagasaki City where Von Siebold stayed.

performed using these primers designed for nested PCR, and the sequence determined (390 bp in length; DDBJ-EMBL-GenBank accession number LC490805) was aligned with 41 sequences (22 haplotypes in type A, 18 in type B, and one in type C) previously reported (Chow et al. 2018b). The nucleotide sequence of 1027-1 was observed to be identical with haplotype JA1 of the A-I clade of *P. paucidens*. Mean K2P distance between 1027-1 and other haplotypes of clade A-I was small ( $0.7 \pm 0.2$  % S.E.) as well as that between 1027-1 and clade A-II ( $1.8 \pm 0.6$  %) (Table 1). In contrast, divergence among three types and between B-I

and B-II was much larger and substantial (4.5 to 8.1 %) (Table 1). Thus, these results indicate the lectotype specimen of *P. paucidens* to be JA1 belonging to the A-I clade of type A. JA1 is the most predominant haplotype in clade A-I and in type A as well, in which 69 individuals were JA1 out of 76 individuals in A-I clade and of 110 type A individuals collected at 101 localities in Japan (Chow et al. 2018b). As shown in Fig. 3, individuals belonging to A-I clade are distributed mainly from the central to southern part of Japan. Historically known as Sakoku Policy, relations and trade between Japan and other countries was

Table 1. Nucleotide sequence divergence (% K2P) (below diagonal) among the type specimen (1027-1) and other types and clades of *Palaemon paucidens*. Above diagonal is standard error.

Type and clade	1027-1	A-I*	A-II	B-I	B-II	C
1027-1	–	0.2	0.6	1.5	1.3	1.5
A-I*	0.7	–	0.5	1.5	1.3	1.5
A-II	1.8	1.8	–	1.3	1.2	1.4
B-I	7.7	7.6	6.9	–	1.0	1.3
B-II	6.6	6.6	5.8	4.5	–	1.2
C	8.0	8.1	8.1	6.6	6.3	–

\*not including JA1 haplotype



Fig. 4. Type specimen (RMNH.CRUS.D.1027) of *Palaemon paucidens* De Haan, 1841 held in Naturalis Biodiversity Center, Leiden, The Netherlands.

severely limited by the Japanese Tokugawa shogunate in the Edo Period. Therefore, survey coverage by Von Siebold must have been quite limited around Nagasaki, and it is reasonable that the *P. paucidens* sample collected by him was of the JA1 haplotype in A-I clade.

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シーボルトが採取し生物多様性センターに収蔵されている  
スジエビ (*Palaemon paucidens*) のタイプ標本について

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スジエビ (*Palaemon paucidens* De Haan, 1844) には遺伝的に異なる 3 タイプ (A、B、C) が発見されていることから複合種であると考えられる。2 世紀近く前にシーボルトが採取しオランダの国立民族学博物館に収蔵されているタイプ標本の 16S rDNA を分析したところ、A タイプのうち JA1 ハプロタイプであることがわかった。JA1 タイプは日本中部から南部にかけて最も多く出現するタイプであり、長崎周辺に限られていたシーボルトの活動範囲とも符合する。

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