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Cosmopolitan or Cryptic Species? A Case Study of *Capitella teleta* (Annelida: Capitellidae)

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Capitella teleta Blake et al., 2009 is an opportunistic capitellid originally described from Massachusetts (USA), but also reported from the Mediterranean, NW Atlantic, and North Pacific, including Japan. This putatively wide distribution had not been tested with DNA sequence data; intraspecific variation in morphological characters diagnostic for the species had not been assessed with specimens from non-type localities, and the species status of the Japanese population(s) was uncertain. We examined the morphology and mitochondrial COI (cytochrome c oxidase subunit I) gene sequences of *Capitella* specimens from two localities (Ainan and Gamo) in Japan. Specimens from Ainan and Gamo differed from *C. teleta* from Massachusetts in methyl-green staining pattern, shape of the genital spines, and shape of the capillary chaetae; we concluded that these characters vary intraspecifically. Species delimitation analyses of COI sequences suggested that worms from Ainan and Massachusetts represent *C. teleta*; these populations share a COI haplotype. The specimens from Gamo may represent a distinct species and comprise a sister group to *C. teleta* s. str.; we refer to the Gamo population as *Capitella* aff. *teleta*. The average Kimura 2-parameter (K2P) distance between *C. teleta* s. str. and *C. aff. teleta* was 3.7%. The COI data indicate that *C. teleta* actually occurs in both the NW Atlantic and NW Pacific. Given the short planktonic larval duration of *C. teleta*, this broad distribution may have resulted from anthropogenic dispersal.

Key words: annelids, sibling species, invasive species, DNA barcoding, PTP, GMYC, ABGD, TCS

INTRODUCTION

Conventional, morphology-based species identification has produced chorological hypotheses that some organisms have a worldwide distribution; these are often referred to as ‘cosmopolitan’ species (e.g., Briggs, 1960; Green, 1972). Since the advent of molecular phylogenetic analysis and barcoding, the status of putative cosmopolitan species has been increasingly testable, especially with recent techniques such as species delimitation analyses using DNA sequence data (e.g., Fontaneto et al., 2015). Some marine invertebrate species have proven to show truly cosmopolitan distributions (e.g., orbinid annelids, Meyer et al., 2008), whereas others have emerged as complexes of morphologically similar species, with examples among gastrotrichs (Kieneke et al., 2012), colonial ascidians (Pérez-Portela et al., 2013), ostracods (Schön et al., 2014), and annelids, including amphinomids and hesionids (Nygren, 2014). Naturally, the issue ‘cosmopolitan species or species complex?’ persists

for putative cosmopolitan species not yet examined with molecular phylogeny.

Annelids in the genus *Capitella* Blainville, 1828 are free-living, marine, benthic animals, occurring around the world (Rouse and Pleijel, 2001). Among 17 species presently recognized (Warren, 1991; Blake et al., 2009; Magalhães and Bailey-Brock, 2012; Silva et al., 2016), *Capitella capitata* (Fabricius, 1780) had long been considered to have a cosmopolitan distribution (Blake, 2009). However, various studies focusing on its anatomy, development, and physiology (e.g., Grassle and Grassle, 1976; Eckelbarger and Grassle, 1983; Gamenick et al., 1998; Linke-Gamenick et al., 2000; Méndez, 2002, 2006) suggested that nominal ‘*C. capitata*’ probably comprises more than 10 cryptic species (Blake et al., 2009). After redescribing *C. capitata* based on topotypic specimens, Blake (2009) concluded that some of these putatively cryptic species can be morphologically distinguished from *C. capitata* sensu stricto. Blake et al. (2009) formally described one such cryptic species, *Capitella* sp. I sensu Grassle and Grassle (1976), as *C. teleta* Blake et al., 2009. None of the nine or more remaining cryptic species has been sufficiently studied morphologically (Grassle and Grassle, 1976; Blake et al., 2009) and no molecular data are available for them.

Five species/subspecies in *Capitella* have been

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reported from Japan: *C. capitata*; *C. capitata japonica* Kitamori, 1960; *C. jonesi* (Hartman, 1959); *C. minima* Langerhans, 1880; and *C. teleta* (e.g., Kitamori, 1960; Imajima and Hartman, 1964; Imajima, 2015; Kanaya et al., 2015). Before *C. teleta* was described in 2009, most *Capitella* specimens from Japan were identified as *C. capitata* (e.g., Kitamori and Funae, 1959; Imajima and Hartman, 1964; Honma et al., 1974; Ueno and Yamamoto, 1982; Tsutsumi and Kikuchi, 1984; Tsutsumi, 1987; Tsutsumi et al., 1991). Tsutsumi and Montani (1993) reported in a personal communication from Judith P. Grassle that *Capitella* specimens from Tomoe Cove (Amakusa, Kyusyu, Japan) were identical with *Capitella* sp. I sensu Grassle and Grassle (1976) from Massachusetts (USA) based on the karyotype, ability to interbreed, and life-history; this taxon has subsequently been reported from Japan in various ecological papers under the name *Capitella* sp. I (e.g., Tsutsumi, 2005; Tsutsumi et al., 2005a, b; Kinoshita et al., 2008) or *C. teleta* (Nishi et al., 2010; Kanaya, 2014; Kanaya et al., 2015). These records, together with reports from California and the Mediterranean (Blake et al., 2009), suggested that *C. teleta* is broadly distributed (Fig. 1), but neither morphological nor molecular data were available to confirm the identity of Japanese materials referable to *C. teleta*.

During a faunal survey of Japanese capitellids, we collected *Capitella* specimens from two localities, Gamo (Miyagi) and Ainan (Ehime) (Fig. 1), that we identified morphologically as *C. teleta*. Here we describe the morphology of these Japanese specimens of *C. teleta*, including the extent of intraspecific variation in some characters as a means of evaluating their taxonomic utility. We also report the results of a species delimitation analyses of mitochondrial COI (cytochrome *c* oxidase subunit I) gene sequences from Japanese *C. teleta* and as well as sequences from other populations available in public databases to test whether *Capitella teleta* in fact is broadly distributed.

MATERIALS AND METHODS

Animals

Worms were collected from a tidal mudflat at Gamo, Miyagi, Japan (38°15'28"N, 141°00'52"E) on 11 June 2013 and 11

November 2014, and from subtidal muddy sediments beneath aquaculture rafts (~40 m depth) near Ainan, Ehime, Japan (32°55'14"N, 132°31'08"E) on 7 November 2013. For 20 specimens collected from Gamo, the anterior portion of the body (including about 20 segments) was fixed in 10% formalin–seawater and later transferred into 70% ethanol for morphological observation; the posterior portion was preserved in 99% ethanol for DNA extraction. For 27 specimens from Ainan, the whole body was fixed in 70% ethanol and then cut into two portions, the anterior for morphological observation and the posterior for DNA extraction. Other specimens from both populations were fixed in 10% formalin–seawater and later transferred to 70% ethanol for morphological observation. Some specimens from both sites were cultured in the laboratory, although these were not preserved as voucher specimens because they disintegrated immediately after their death.

Morphological observations

Morphological observations were made under an SMZ 1500 stereomicroscope (Nikon, Japan), BX51 compound light microscope (Olympus, Japan), and S-3000N scanning electron microscope (SEM; Hitachi, Japan). Photographs were taken with a D5200 digital camera (Nikon) attached to the SMZ 1500 and BX51 by NYPIXS2-3166, HY1S-FA, and NY1S-1501750 adapters (Micro-net, Japan), with external lighting by a pair of Hikaru Komachi Di strobes (Morris, Japan). Morphological observations including methyl-green staining were performed as described by Tomioka et al. (2013). Morphological terminology follows Blake et al. (2009). The specimens from this study have been deposited in the Invertebrate Collection of the Hokkaido University Museum, Sapporo, Japan (ICHUM).

DNA extraction, PCR, and sequencing

Total DNA was extracted from the posterior portion of worms with a DNeasy Blood & Tissue Kit (Qiagen, USA). Primers pair LCO-1490 and HCO-2198 (Folmer et al., 1994), was used in PCR amplification and sequencing for two outgroup taxa: *Heteromastus* sp. from Takehara, Japan, and *Capitella* sp. from Mombetsu, Japan. Because multiple sequences were detected in the latter species, PCR products were cloned using the pGEM-T Easy Vector System (Promega, USA) following Inoue et al. (1990), and then PCR amplified and sequenced with a pair of M13 primers (Oetting et al., 1995). Based on the sequence of *Capitella* sp. from Mombetsu, a pair of specific primers were designed, *Capitella_COI_F* (5'-GGAATTTGAGGTGGGCTTGT-3') and *Capitella_COI_R* (5'-CAC-CACCACCAGTATTATCA-3'), using Primer3Plus (Rozen and Skaletsky, 2000); this primer set was used for PCR amplification

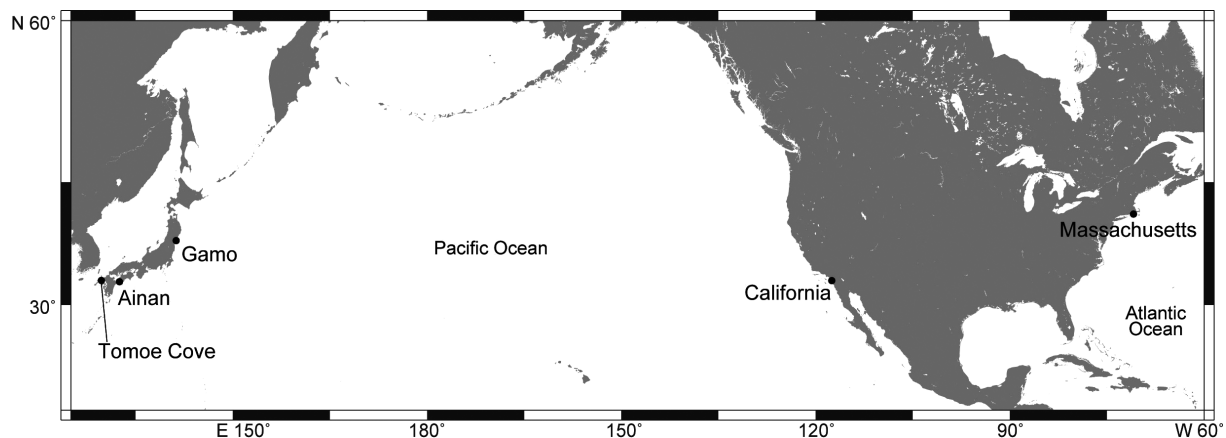


Fig. 1. Map showing sampling sites in Japan and three previously reported sites (Blake et al., 2009) for *Capitella teleta* Blake et al., 2009 (Mediterranean records omitted).

Table 1. List of specimens treated in this study, with the museum catalog number, state of preservation, sampling locality, sexuality, DDBJ/GenBank accession number for COI sequences, and references. Asterisk (*) indicates sequences derived from gut contents of the spotted catfish *Arius maculatus* (Thunberg, 1792).

Specimen name	Accession number of DDBJ/GenBank	Catalog number (ICHUM)	State of preservation	Sampling locality	Sexuality	References
Ainan 1	LC120627	5149	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 2	LC120628	5150	in 70% ethanol	Ainan, Ehime, Japan	female	this study
Ainan 3	LC120629	5151	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 4	LC120630	5152	mounted on slides	Ainan, Ehime, Japan	female	this study
Ainan 5	LC120631	5153	mounted on SEM stub	Ainan, Ehime, Japan	female	this study
Ainan 6	LC120632	5154	in 70% ethanol	Ainan, Ehime, Japan	female	this study
Ainan 7	LC120633	5155	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 8	LC120634	5156	mounted on slides	Ainan, Ehime, Japan	male	this study
Ainan 9	LC120635	5157	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 10	–	5158	mounted on SEM stub	Ainan, Ehime, Japan	male	this study
Ainan 11	–	5159	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 12	–	5160	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 13	–	5161	mounted on slides	Ainan, Ehime, Japan	male	this study
Ainan 14	–	5162	in 70% ethanol	Ainan, Ehime, Japan	female	this study
Ainan 15	–	5163	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 16	–	5164	in 70% ethanol	Ainan, Ehime, Japan	female	this study
Ainan 17	–	5165	in 70% ethanol	Ainan, Ehime, Japan	female	this study
Ainan 18	–	5166	mounted on SEM stub	Ainan, Ehime, Japan	female	this study
Ainan 19	LC120636	5167	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 20	LC120637	5168	in 70% ethanol	Ainan, Ehime, Japan	female	this study
Ainan 21	LC120638	5169	mounted on slides	Ainan, Ehime, Japan	male	this study
Ainan 22	LC120639	5170	mounted on SEM stub	Ainan, Ehime, Japan	male	this study
Ainan 23	–	5171	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 24	LC120640	5172	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 25	–	5173	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 26	–	5174	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Ainan 27	–	5175	in 70% ethanol	Ainan, Ehime, Japan	male	this study
Gamo 1	LC120641	5129	in 70% ethanol	Gamo, Miyagi, Japan	male	this study
Gamo 2	LC120642	5130	mounted on slides	Gamo, Miyagi, Japan	male	this study
Gamo 3	LC120643	5131	in 70% ethanol	Gamo, Miyagi, Japan	male	this study
Gamo 4	LC120644	5132	in 70% ethanol	Gamo, Miyagi, Japan	female	this study
Gamo 5	LC120645	5133	in 70% ethanol	Gamo, Miyagi, Japan	male	this study
Gamo 6	LC120646	5134	mounted on SEM stub	Gamo, Miyagi, Japan	male	this study
Gamo 7	LC120647	5135	in 70% ethanol	Gamo, Miyagi, Japan	male	this study
Gamo 8	–	5136	in 70% ethanol	Gamo, Miyagi, Japan	male	this study
Gamo 9	LC120648	5137	mounted on slides	Gamo, Miyagi, Japan	female	this study
Gamo 10	–	5138	in 70% ethanol	Gamo, Miyagi, Japan	female	this study
Gamo 11	–	5139	in 70% ethanol	Gamo, Miyagi, Japan	female	this study
Gamo 12	–	5140	mounted on SEM stub	Gamo, Miyagi, Japan	female	this study
Gamo 13	–	5141	in 70% ethanol	Gamo, Miyagi, Japan	female	this study
Gamo 14	–	5142	in 70% ethanol	Gamo, Miyagi, Japan	male	this study
Gamo 15	–	5143	mounted on slides	Gamo, Miyagi, Japan	male	this study
Gamo 16	LC120649	5144	in 70% ethanol	Gamo, Miyagi, Japan	male	this study
Gamo 17	–	5145	in 70% ethanol	Gamo, Miyagi, Japan	female	this study
Gamo 18	LC120650	5146	in 70% ethanol	Gamo, Miyagi, Japan	female	this study
Gamo 19	–	5147	in 70% ethanol	Gamo, Miyagi, Japan	female	this study
Gamo 20	–	5148	in 70% ethanol	Gamo, Miyagi, Japan	female	this study
<i>Capitella</i> sp.	LC120651	–	in 70% ethanol	Mombetsu, Hokkaido, Japan	female	this study
<i>Capitella</i> sp.	LC120652	–	in 70% ethanol	Mombetsu, Hokkaido, Japan	female	this study
<i>Capitella</i> sp.	LC120653	–	in 70% ethanol	Mombetsu, Hokkaido, Japan	female	this study
<i>C. teleta</i>	–	–	–	Woods Hole, Massachusetts, USA	–	Blake et al. (2009)
<i>C. capitata</i>	GU672406	–	–	Gordon Point, Churchill, Canada	–	Carr et al. (2011)
<i>C. capitata</i>	GU672407	–	–	Gordon Point, Churchill, Canada	–	Carr et al. (2011)
<i>C. capitata</i>	HQ023469	–	–	Button Bay, Churchill, Canada	–	Carr et al. (2011)
<i>C. capitata</i>	HQ023470	–	–	Button Bay, Churchill, Canada	–	Carr et al. (2011)
<i>C. capitata</i>	HQ023471	–	–	Bird Cove, Churchill, Canada	–	Carr et al. (2011)
<i>C. capitata</i>	HQ023472	–	–	Bird Cove, Churchill, Canada	–	Carr et al. (2011)
<i>C. capitata</i>	HQ023473	–	–	Button Bay, Churchill, Canada	–	Carr et al. (2011)
<i>C. capitata</i>	HQ023474	–	–	Button Bay, Churchill, Canada	–	Carr et al. (2011)
<i>C. capitata</i>	JX676137*	–	–	India	–	unpublished
<i>C. capitata</i>	JX676150*	–	–	India	–	unpublished
<i>C. capitata</i>	JX676169*	–	–	India	–	unpublished
<i>C. capitata</i>	JX676171*	–	–	India	–	unpublished
<i>C. capitata</i>	JX676173*	–	–	India	–	unpublished
<i>C. capitata</i>	JX676174*	–	–	India	–	unpublished
<i>C. capitata</i>	JX676178*	–	–	India	–	unpublished
<i>C. capitata</i>	JX676179*	–	–	India	–	unpublished
<i>C. capitata</i>	KF737175	–	–	India	–	unpublished
Annelida sp.	KP254815	–	–	Indian River Lagoon, Florida, USA	–	Leray and Knowlton (2015)
Polychaeta sp.	KP254366	–	–	eastern shore of Virginia, USA	–	Leray and Knowlton (2015)
Polychaeta sp.	KP254809	–	–	eastern shore of Virginia, USA	–	Leray and Knowlton (2015)
<i>Heteromastus</i> sp.	LC120654	–	in 70% ethanol	Takehara, Hiroshima, Japan	uncertain	this study
<i>Mediomastus opertaculeus</i>	AB794988	4375	in 70% ethanol	Abashiri, Hokkaido, Japan	uncertain	Tomioka et al. (2013)

and sequencing for specimens from Gamo and Ainan.

PCRs were performed using an iCycler thermal cycler (BioRad, USA) in 10- μ l reaction volumes containing 1 μ l of total DNA template, 1 μ l of 10 \times Ex Taq buffer (TaKaRa Bio, Japan), 25 mM of each dNTP, 10 μ M of each primer, and 2.5 U of TaKaRa Ex Taq DNA polymerase (5 U/ μ l; TaKaRa Bio) in deionized water. Thermal cycling conditions were 95°C for 90 s; 35 cycles of 95°C for 30 s, 50°C (for LCO-1490/HCO-2198) or 55°C (for *Capitella_COI_F/* *Capitella_COI_R*) for 30 s, and 72°C for 1 min; and 72°C for 7 min. PCR products were purified following the method of Boom et al. (1990) with some modifications (Kobayashi et al., 2009). Terminator reactions were done with a BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, USA) following the manufacturer's protocol. Sequencing was performed with a 3730 DNA Analyzer (Applied Biosystems, USA). Sequences were checked and assembled by using MEGA 5.2.2 (Tamura et al., 2011).

In all, sequences (536 bp) were determined for 28 individuals (10 from Gamo, 14 from Ainan, three for *Capitella* sp. from Mombetsu, and one for *Heteromastus* sp. from Hiroshima) and have been deposited in DDBJ (accession numbers LC120627–LC120654).

Phylogenetic tree construction and species delimitation analyses

In addition to newly generated sequences, the following COI sequences from public databases were included in the analyses:

Mediomastus opertaculeus Tomioka et al., 2013 (GenBank accession no. AB794988); 23 sequences in GenBank that showed identity scores greater than 80% in BLAST searches (Altschul et al., 1997) using the sequences from Gamo and Ainan specimens [eight of the 23 sequences—JX676137, JX676150, JX676169, JX676171, JX676173, JX676174, JX676178, and JX676179—appeared to be derived from gut contents of the spotted catfish, *Arius maculatus* (Thunberg, 1792) in India (Kumar et al., 2011)]; and *C. teleta* from the Genome Portal of the Department of Energy Joint Genome Institute (<http://genome.jgi.doe.gov/>; deposited as *Capitella* sp. I ESC-2004 with Protein ID = 228595). The COI sequence given in Blake et al. (2009, p. 32) lacks a portion that was erroneously identified as an 'intron'. Table 1 summarizes information for all sequences used in the analyses.

MUSCLE (Edgar, 2004a, b) was used to align the sequences. The optimal nucleotide substitution model for maximum likelihood (ML) and Bayesian inference (BI) analyses was selected with MEGA 5.2.2, using the Akaike information criterion (Akaike, 1974); the GTR+I+G model (Tavaré, 1986) was optimal for both analyses. The ML analysis was conducted in MEGA 5.2.2, with nodal support values obtained through ML analyses of 10,000 bootstrap pseudoreplicates (Felsenstein, 1985). The BI analysis was performed with BEAST ver. 1.8.2 (Drummond et al., 2012). A Markov chain Monte Carlo analysis (MCMC) was simulated for one billion generations and sampled every 100 generations; burn-in was set to 10%. Kimura's (1980) 2-parameter (K2P) distances were calculated with

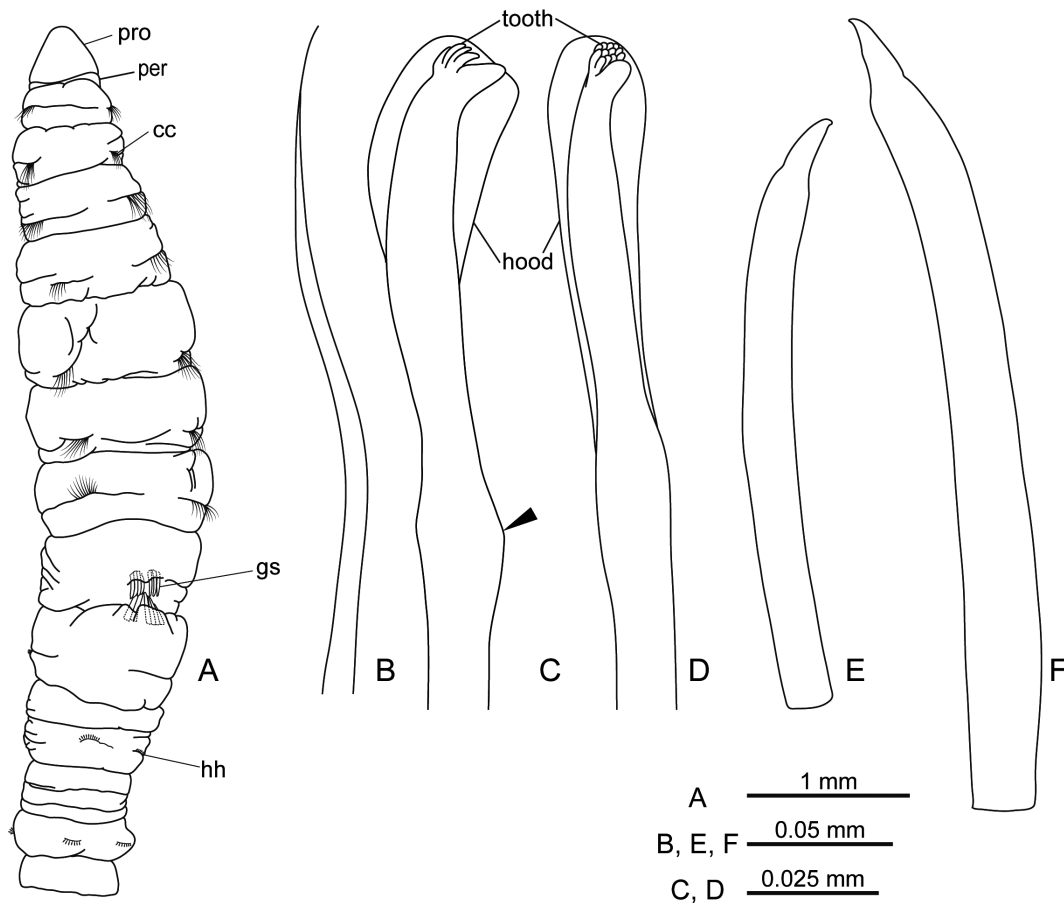


Fig. 2. *Capitella teleta* Blake et al., 2009 (Ainan population). (A) Male (ICHUM 5167, Ainan 19). (B–F) Male (ICHUM 5169, Ainan 21). (A) Anterior end of body, dorsal view. (B) Notopodial capillary chaeta from segment 8. (C) Notopodial hooded hook from segment 12, right view. (D) Notopodial hooded hook from segment 12, frontal view. (E) Genital spine from segment 8, left view. (F) Genital spine from segment 9, left view; arrowhead indicates node of hooded hook. Abbreviations: cc, capillary chaeta; gs, genital spine; hh, hooded hook; per, peristomium; pro, prostomium.

MEGA 5.2.2.

Five models were used for the species delimitation analyses: Poisson tree processes (PTP) (Zhang et al., 2013); Bayesian PTP

(bPTP) (Zhang et al., 2013); generalized mixed Yule coalescent (GMYC) (Pons et al., 2006; Fujisawa and Barraclough, 2013); automatic barcode gap discovery (ABGD) (Puillandre et al., 2012); and

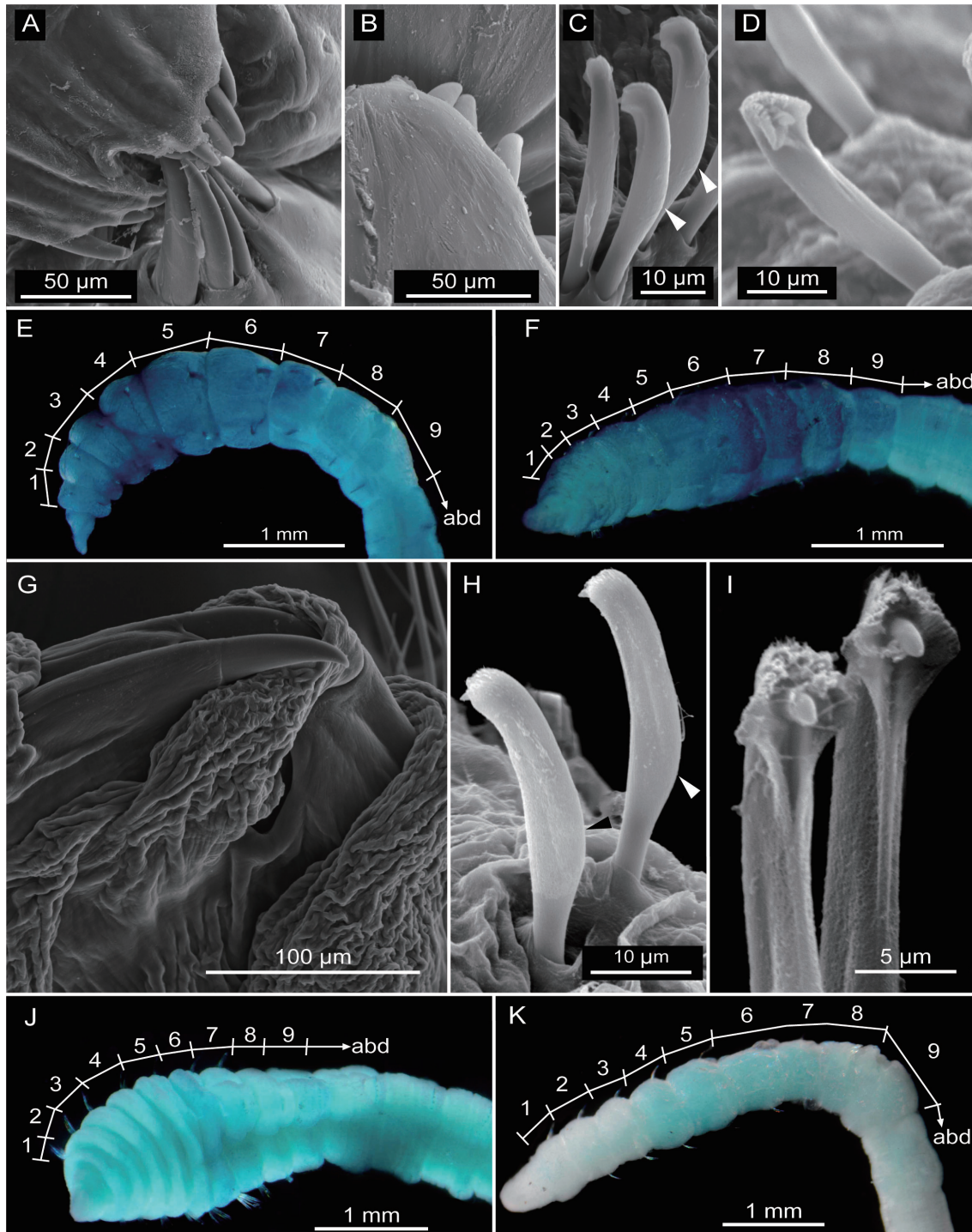


Fig. 3. Photographs of *Capitella teleta* Blake et al., 2009 (Ainan population) (A–F) and *Capitella* aff. *teleta* (Gamo population) (G–K). (A) Male (ICHUM 5170, Ainan 22). (B–D) Male (ICHUM 5158, Ainan 10). (E) Male (ICHUM 5169, Ainan 21). (F) Female (ICHUM 5150, Ainan 2). (G–I) Male (ICHUM 5134, Gamo 6). (J) Male (ICHUM 5129, Gamo 1). (K) Male (ICHUM 5144, Gamo 16). (A) Genital spines on segments 8–9. (B) Genital spine on segment 9. (C) Notopodial hooded hooks on segment 8, posterior view. (D) Neuropodial hooded hooks on segment 25, frontal view. (E) Methyl-green staining pattern in male, anterior end of body. (F) Methyl-green staining pattern in female, anterior end of body. (G) Genital spines on segments 8–9. (H) Notopodial hooded hooks on segment 9, posterior view. (I) Notopodial hooded hooks on segment 20, frontal view. (J) Methyl-green staining pattern in which the whole body was uniformly stained. (K) Methyl-green staining pattern in which segments 4–7 were stained; arrowheads indicate enlargement of shaft. (E–F), (J–K) with the segment numbers and segmental boundaries labeled (abd, abdomen).

TCS (Clement et al., 2000). The PTP/bPTP analyses were performed with the ML tree in a web-based interface (<http://species.h-its.org/ptp/>), using the following default parameters: No. MCMC generations = 100,000; thinning = 100; burn-in = 0.1, seed = 123. The GMYC analysis was performed in R 3.0.3 (R Core Team, 2014) with the Splits package (<http://splits.r-forge.r-project.org/>), using an ultrametric tree constructed with BEAST ver. 1.8.2 as the input tree. The ABGD analysis was carried out with the aligned sequence dataset and performed in a web-based interface (<http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html>) with parameters suitable for COI sequences suggested by previous studies (e.g., Puillandre et al., 2012; Tang et al., 2012): Pmin, 0.001; Pmax, 0.01; steps, 10; X, 1.5; Nb bins, 20. The TCS analysis was conducted using the aligned sequence dataset, with the parsimony connection limit set at 95%.

RESULTS

Morphological accounts

Ainan population
***Capitella teleta* Blake et al., 2009**
(Figs. 2, 3A–F, Table 1)

Material examined. 27 specimens (ICHUM 5149–5175) (Table 1).

Sequences. Partial COI sequences (536 bases) from 14 specimens (ICHUM 5149–5157, 5167–5170, 5172; DDBJ accession numbers LC120627–LC120640).

Description (based mainly on one fixed male specimen, ICHUM 5167, Ainan 19). Anterior 16 segments 4.39 mm in length and 0.66 mm in maximum width (segment 6). All segments cylindrical. Branchiae absent.

Prostomium (Figs. 2A, 3E, F) rounded conical, without palpode, laterally with/without pair of red eyespots visible only in living specimens. Peristomium fused with prostomium, not segmental, achaetigerous.

Thorax with nine biannulate segments (Figs. 2A, 3E, F). Segments 1–7 with non-winged, whip-like capillary noto- and neurochaetae (Fig. 2A, B), 3–7 per fascicle. Segments 8–9 with hooded hooks on neuropodia, 3–7 per fascicle; no chaetae/hooks on notopodia; with a pair of genital-spine fascicles mid-dorsally, 2–4 per fascicle. Tip of genital spines beak-shaped in 17 of 18 males observed (e.g., ICHUM 5170, Ainan 22; Fig. 3A); rounded in the remaining one (ICHUM 5158, Ainan 10; Fig. 3B). Spines shorter and thinner on segment 8 than on segment 9.

Abdominal segments more wrinkled than thoracic segments (Figs. 2A, 3E, F), with hooded hooks on noto- and neuropodia (Fig. 2A), 2–7 per fascicle; parapodial ridges

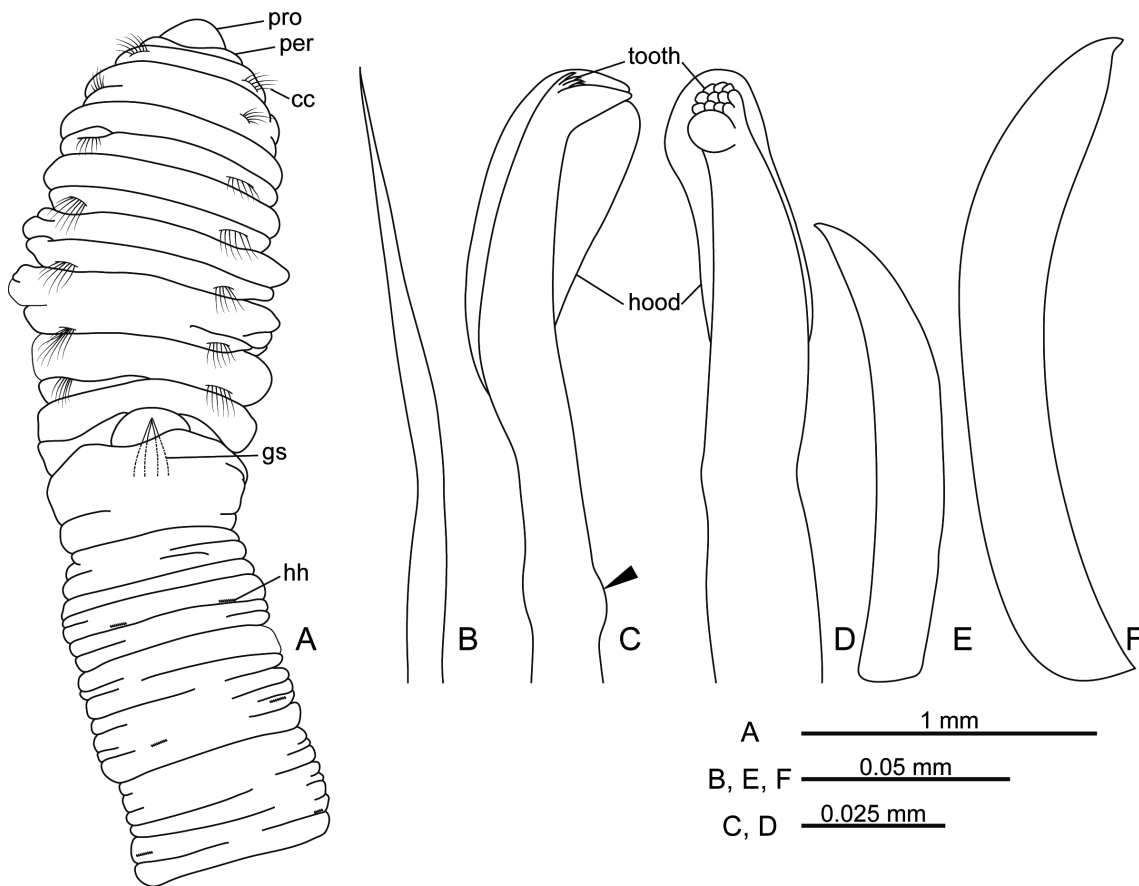


Fig. 4. *Capitella* aff. *teleta* Blake et al., 2009 (Gamo population). (A) Male (ICHUM 5129; Gamo 1). (B–F) Male (ICHUM 5144; Gamo 16). (A) Anterior end of body, dorsal view. (B) Notopodial capillary chaeta from segment 5. (C) Notopodial hooded hook from segment 11, right view. (D) Neuropodial hooded hook from segment 13, frontal view. (E) Genital spine from segment 8, right view. (F) Genital spine from segment 9, right view; arrowhead indicates node of hooded hook. Abbreviations: cc, capillary chaeta; gs, genital spine; hh, hooded hook; per, peristomium; pro, prostomium.

absent. Hooded hooks with long main fang (Fig. 2C, D), 3–4 rows of teeth on main fang, and 3–4 teeth per row; hood flared (Fig. 3D); shaft slightly enlarged like manubrium (Fig. 3C, arrowheads), with node near base (Fig. 2C, arrowhead).

Transition from thorax to abdomen marked by alternation in shape of segments (more wrinkled in anterior abdomen).

Females generally similar to males, except segments 8–9 with non-winged, whip-like capillary chaetae or hooded hooks on notopodia, 2–7 per fascicle; genital spines absent.

Pygidium round, without caudal cirrus and anal plate (confirmed in intact specimens, e.g., ICHUM 5166, Ainan 18).

Methyl-green staining. Whole body stained in both sexes (Fig. 3E, F), but in females, narrow band of numerous dark spots appeared in posterior region of one or more segments among segments 6–9 (Fig. 3F).

Gamo population
***Capitella* aff. *teleta* Blake et al., 2009**
(Figs. 3G–K, 4, Table 1)

Material examined. 20 specimens (ICHUM 5129–5148) (Table 1).

Sequences. Partial COI sequences (536 bases) from 10 specimens (ICHUM 5129–5135, 5137, 5144, 5146; DDBJ accession numbers LC120641–LC120650).

Description (based mainly on one fixed male specimen, ICHUM 5129, Gamo 1). Anterior 26 segments 3.73 mm in length and 0.48 mm in maximum width (segment 4). All segments cylindrical. Branchiae absent.

External morphological characters, including number of thoracic segments (Figs. 3J, K, 4A) and shape of chaetae and hooded hooks (Figs. 3H, I, 4B–D), were identical to those in specimens from Ainan, but genital spines (Figs. 3G, 4E, F) tipped like a beak in all specimens observed.

Methyl-green staining. Two patterns were observed (Fig. 3J, K): in 15 specimens, the whole body stained mildly and uniformly (Fig. 3J); in other five specimens, only segments 4–7 stained (Fig. 3K).

Molecular phylogeny, species delimitation analyses, and K2P distances

The aligned dataset was 536 bases long, without indels. The ML tree showed seven strongly supported clades (Fig. 5, Clades 1–7), most supported with 100% bootstrap value (BS) and 1.00 posterior probability (PP), except for Clade 2 (99% BS, 1.00 PP) and Clade 4 (consisting of a single sequence). Clade 1 contained individuals from the Ainan

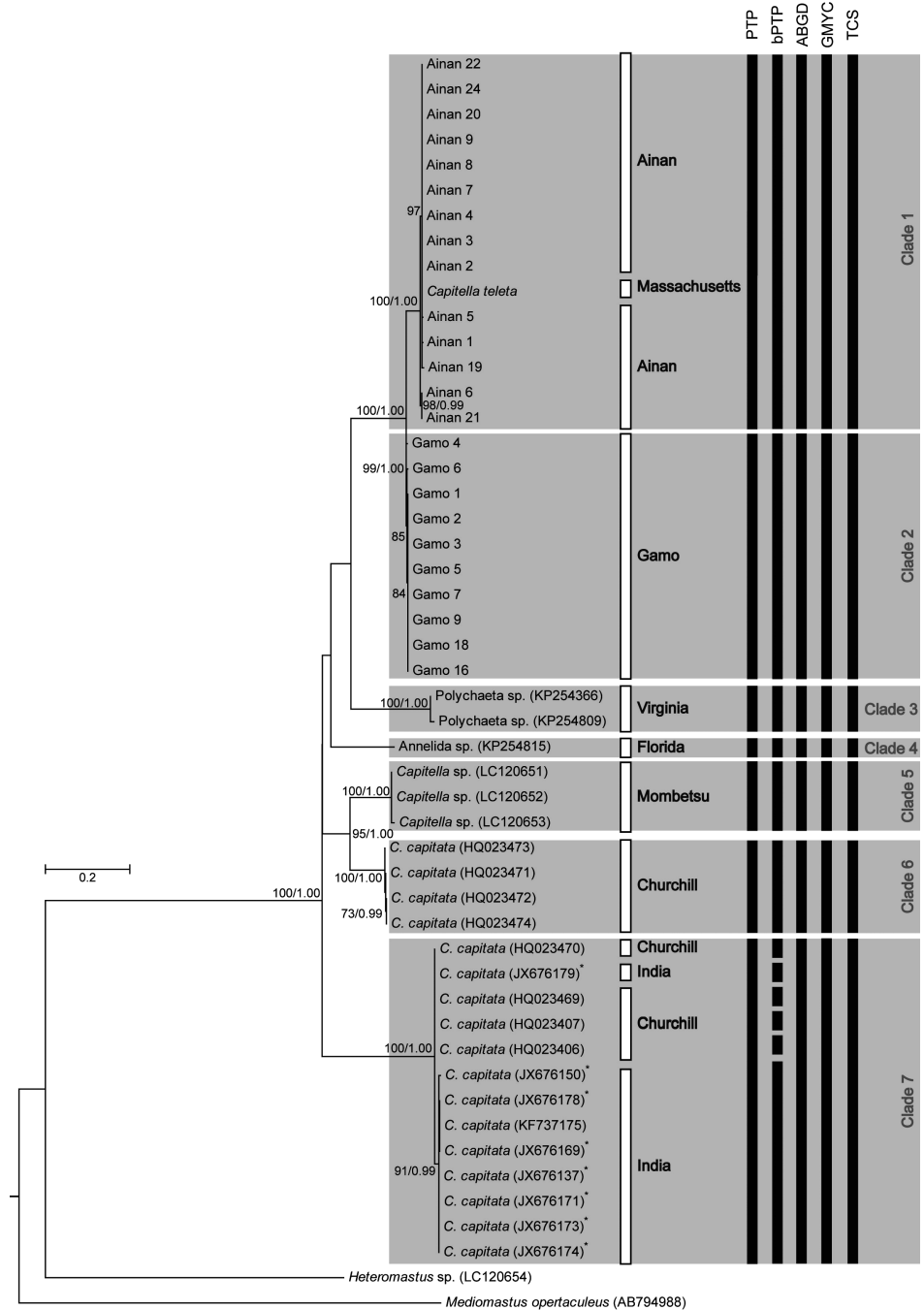


Fig. 5. Maximum-likelihood tree resulting based on COI gene sequences. Numbers near nodes indicate maximum-likelihood bootstrap support value in percent (> 70%) and posterior probability from Bayesian inference (> 0.90). White bars to right of tree indicate sampling sites; black bars indicate results of five species delimitation analyses: PTP, bPTP, ABGD, GMYC, TCS. Asterisk (*) indicates sequences derived from gut contents of the spotted catfish *Arius maculatus* (Thunberg, 1792).

Table 2. K2P distances within (shaded, along diagonal) and between the clades in Fig. 5. For each comparison, the upper row gives the range of values and the lower row the average value.

	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6	Clade 7
Clade 1	0.000–0.011 0.003						
Clade 2	0.033–0.041 0.037	0.000–0.006 0.002					
Clade 3	0.205–0.212 0.206	0.189–0.197 0.191	0.000–0.006 0.004				
Clade 4	0.179–0.184 0.188	0.162–0.167 0.167	0.182–0.187 0.183	– –			
Clade 5	0.182–0.194 0.188	0.162–0.170 0.166	0.184–0.196 0.189	0.193–0.201 0.197	0.008 0.008		
Clade 6	0.170–0.180 0.174	0.191–0.206 0.203	0.121–0.132 0.126	0.173–0.178 0.176	0.207–0.221 0.213	0.000–0.004 0.003	
Clade 7	0.196–0.211 0.204	0.191–0.206 0.201	0.197–0.207 0.202	0.198–0.206 0.202	0.199–0.214 0.207	0.210–0.223 0.217	0.000–0.013 0.003

population and *C. teleta* from Massachusetts (type locality). Clade 2 contained individuals from the Gamo population. Clades 3–5 were formed by sequences from Virginia (USA), Florida (USA), and Mombetsu (Japan), respectively. Clade 6 consisted of four sequences from Churchill (Manitoba, Canada). The other sequences from Churchill formed Clade 7, together with sequences from a population in India that included sequences recovered from gut contents of the spotted catfish *Arius maculatus*.

All species delimitation analyses except bPTP detected seven 'species', which were congruent with the seven clades recovered by ML/BI (Fig. 5). The bPTP analysis delimited six 'species' in Clade 7, as shown in Fig. 5. All methods indicated that the Ainan population and *C. teleta* from Massachusetts (Fig. 5, Clade 1) belong to the same species, and that the Gamo population (Fig. 5, Clade 2) represents a distinct species from the former.

Table 2 gives K2P distances within and between clades. K2P distances were 0.000–0.011 within Clade 1, 0.000–0.006 within Clade 2, and 0.033–0.041 between Clades 1 and 2; distances were much higher (0.126–0.217) in pairwise comparisons among other clades (Table 2).

DISCUSSION

Our observations on the external morphology of specimens from Gamo and Ainan are consistent with the original description of *C. teleta* from Massachusetts by Blake et al. (2009), except for the methyl-green staining pattern, shape of genital spines, and shape of capillary chaetae. The methyl-green staining pattern can differ among individuals and between the sexes within the same population (Fig. 3E, F, J, K). The shape of the genital spines can also differ among individuals within a single population (Fig. 3A, B). The shape of capillary chaetae differs between Japanese populations (without wing; Figs. 2B, 4B) and *C. teleta* from Massachusetts (unilimbate; Blake et al., 2009), but our COI data indicate that the Ainan and Massachusetts populations are undoubtedly conspecific (Fig. 5, Clade 1). These morphological characters are variable within a species, and thus should be used with caution in the species-level taxonomy of *Capitella*. Our species delimitation analyses based on the COI sequences currently available suggested that worms

from the Ainan population were *C. teleta*, with some individuals from Ainan and Massachusetts sharing the haplotype that was included in the original description of the species (Blake et al., 2009).

The Gamo population should be referred to as *Capitella* aff. *teleta* until its taxonomic identity can be fully resolved with additional sequence data and other biological information. In our phylogenetic analyses, sequences from Gamo comprised the sister taxon to *C. teleta* s. str. Although the

Gamo population may represent a distinct biological entity (Fig. 5), we refrained from formally describing it as a new species or applying an existing name other than *C. teleta* to it. It is because we cannot rule out the possibility that intermediate sequences might fill the gap between Clades 1 and 2 if specimens from additional sampling are included in future analyses, especially considering the genetic distance between the two (average K2P distance, 3.7%; Table 2), which is relatively smaller than the value that are often greater than 15% between cryptic polychaete species (Nygren, 2014).

Sequences in GenBank from specimens identified as *C. capitata* appeared in two clades, and our analyses suggest they represent two different species (Fig. 5, Clades 6 and 7). There are more than 30 potentially available species-group names in *Capitella* (Read, 2015), including *C. capitata* s. str. from Greenland (type locality); to apply names to these and other divergent clades (Clades 3–7) evident in our tree, we would need to designate a COI sequence from reliably identified material for each of the nominal species/subspecies, especially for the oldest name *C. capitata* (Clades 6 and 7, Fig. 5).

Our analyses confirmed that *C. teleta* occurs in both the NW Atlantic and NW Pacific (Fig. 1), as was previously suggested (Tsutsumi and Montani, 1993; Blake et al., 2009). Considering the shared COI haplotype between Massachusetts and Ainan, and the short planktonic larval duration (several hours; Blake et al., 2009), the broad distribution of *C. teleta* is likely due to anthropogenic dispersal, e.g., via ships' ballast water (e.g. Chu et al., 1997; Dorgham and Hamdy, 2015). This may also be the case for individuals in Clade 7 (Fig. 5) from Churchill (Canada) and India.

In this study, we applied species delimitation analyses to capitellid worms, detecting a potential cryptic species (the Gamo sister-clade to *C. teleta*) and cosmopolitan species (i.e., *C. teleta*) in *Capitella*. The genus *Capitella* is a good potential target to unravel the cryptic species that have been overlooked. For instance, there are still a number of unnamed cryptic species (Grassle and Grassle, 1976; Eckelbarger and Grassle, 1983; Gamenick et al., 1998; Linke-Gamenick et al., 2000; Méndez, 2002, 2006; Blake, 2009), some of which might be a true cosmopolitan species

at the same time. The issue 'cosmopolitan species or species complex?' remains in the taxonomy of many annelid groups, including Capitellidae, and other marine invertebrates.

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