

## Northernmost discovery of Bathynellacea (Syncarida: Bathynellidae) with description of a new species of *Pacificabathynella* from Alaska (USA)

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### ABSTRACT

A new species of the genus *Pacificabathynella* Schminke and Noodt, 1988 is described from groundwaters of Alaska (USA). This is the first record of Bathynellacea Chappuis, 1915 from the far north of America. *Pacificabathynella* has hitherto been known only from the states of California (one species) and Montana (three species). *Pacificabathynella* and *Paradoxibathynella* Serban, 2000 are the only genera that show sexual dimorphism in thoracopod VI. *Pacificabathynella yupik* sp. nov. has several unique features within the genus: antenna eight-segmented; antennule only slightly longer than the antenna; the setal formula of the maxilla (7/3/7/5); without seta on the endopod of the male thoracopod VIII; five spines on the endopod of the uropod and the endopod as long as the sympod. The new species further shows slight differences in the antennula, pars molaris of the mandible, in thoracopod I–VII and in thoracopod VIII of males and females with the other species of the genus. Sequences of mitochondrial DNA (mtDNA) cytochrome c oxidase I (COI) of five specimens of the new species were obtained to complete the morphological description with molecular data.

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## Introduction

The Family Bathynellidae Grobben, 1905 in North America includes only seven known species belonging to two genera: *Bathynella* Vejdovsky, 1882, with three species (one in Colorado and two others in California) and *Pacificabathynella* Schminke and Noodt, 1988, with four species, one in San Francisco, California and three in Montana (Camacho, Newell, et al., 2009).

This family is widespread throughout the world, with 28 genera and 102 species (Camacho 2015) but is poorly known in North America. Current taxonomy of the genus *Bathynella* is not satisfactory, and includes more than 50 species and subspecies (Camacho 2006) distributed throughout the world, but few have been properly studied.

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**Table 1.** Characteristics of the two genera of the Tribe Pacificabathynellini Serban, 2000. *Pacificabathynella* Schminke and Noodt, 1988 and *Paradoxibathynella* Serban, 2000. Abbreviations: A.I, antennule; A.II, Antenna; Th, thoracopod. (Modified after Camacho, Newell, et al., 2009).

|                               | <i>Pacificabathynella</i>       | <i>Paradoxibathynella</i> |
|-------------------------------|---------------------------------|---------------------------|
| A.I: segments                 | 7                               | 7                         |
| A.II: segments                | 7–8                             | 7                         |
| A.I/A.II                      | A.I << A.II/ A.I>A.II/ A.I=A.II | A.I < A.II/ A.I= A.II     |
| Md: teeth of the pars molaris | two dentated lobes              | 6 (5 + 1 big)             |
| Epipod Th. I                  | absent                          | absent                    |
| Epipod Th. II                 | absent/present                  | present                   |
| Exopod of the Th I to VII     | 5–6 setae                       | 5 setae                   |
| Sexual dimorphism on Th. VI   | present                         | present                   |
| Th VIII male                  |                                 |                           |
| lobes in the penial region    | 3                               | 4                         |
| prominence in the basipod     | present                         | absent                    |
| exopod                        | 1-segmented                     | 1-segmented               |
| endopod                       | 1-segmented                     | 1-segmented               |
| Th VIII female                |                                 |                           |
| coxa                          | 1–2 plumose setae               | 3 smooth setae            |
| basipod                       | 1–2 setae                       | 1 seta                    |
| epipod                        | present                         | present                   |
| exopod: number apical seta    | 2                               | 4                         |
| endopod: number apical seta   | 1–2                             | 2                         |
| Uropod: sympod                | 5–8 spines                      | 4 spines                  |
| endopod                       | 4–8 strong spines               | 4 strong spines           |
| First pleopods: setae         | 1–6/1–7                         | 1–8/1–9                   |
| Male Min.-Max. length         | 0.53–2.07                       | 1.10–1.30                 |
| Female Min.-Max. length       | 0.51–1.78                       | 0.80–1.38                 |

In 1988, *Pacificabathynella* was the only known genus with sexual dimorphism in thoracopod VI of the male. Serban (2000) described a new genus from Japan, *Paradoxibathynella*, which also has similar sexual dimorphism, to include *Bathynella yezoensis* (Uéno, 1954) considered as belonging to *Pacificabathynella* by Schminke and Noodt, 1988. Serban (2000) created the new tribe Pacificabathynellini to include the two genera with sexual dimorphic thoracopod VI.

The new species described in this paper undoubtedly belongs to the genus *Pacificabathynella* (see Table 1).

Normally, unique combinations of the morphological characters can define genera and species, but individually these characters do not seem to delineate species in this special groundwater crustacean group. Due to extreme morphological simplification and convergence in Bathynellacea Chappuis, 1915 (Camacho et al. 2013), in some cases morphology alone is not enough to identify closely related species and this causes difficulties in the generic assignment of species as well. So, it now appears essential to obtain DNA sequences for authentic comparison of closely allied species. This is particularly true in the case of the Bathynellidae, where a combined molecular and morphological approach is strongly recommended for any future taxonomic research. In this paper we have followed both these approaches to characterise *Pacificabathynella yupik* sp. nov. from the USA. The sequences of mitochondrial DNA (mtDNA) cytochrome c oxidase I (COI) were obtained from five specimens of the new species to supplement the morphological description and also have reference characters in our library of genes that allow future comparisons. The nuclear 18S rRNA is usually used to find relationships

between genera of a family. The small subunit (SSU) 18S rRNA gene is one of the most frequently used genes in phylogenetic studies and an important marker for random target PCR in environmental biodiversity screening (Chenuil, 2006), but unfortunately we failed to obtain a long fragment of 18S.

With this and other recent discoveries from glaciated western Montana (i.e. Camacho, Stanford, et al. 2009), the true diversity of the group in North America has begun to emerge. For the first time a species is found at a northernmost point in the boreal hemisphere, above latitude 60 (60.34520° N). *Antrobathynella stammeri stammeri* (Jakobi, 1954) was found in the River Lathkill, Derbyshire, England. (54.1896195° N/-2.4015749 W) and *Baikalobathynella magna* (Bazikalova, 1954) was found in the Lake Baikal, Angara river bassin (53.5600973° N/108.1646634° E), both only slightly above latitude 50.

## Material and methods

The specimens studied come from one sample collected in a locality in western Alaska (USA): gravel bar, Kwethluk River, a tributary of the Kuskokwim River.

### Study area

The Kwethluk River is a fifth-order tributary to the Kuskokwim River within the Yukon Delta National Wildlife Refuge, Alaska, USA. The Kwethluk originates in the Eek and Crooked Mountains and flows northwest for 222 km across an expansive tundra plain underlain by permafrost (Johnson and Hartman 1969). The catchment is 3367 km<sup>2</sup>. The region has a subarctic climate with temperatures ranging from above 12°C in summer to -12°C as a winter average (Alt 1977). The river supports a forested riparian corridor on expansive alluvial flood plains underlain by shallow alluvial aquifers that moderate temperatures and prevent permafrost. Woody vegetation consists of willow (*Salix* spp.), alder (*Alnus* spp.), cottonwood (*Populus* sp.), and spruce (*Picea glauca*) stands, with substantial influence by beaver (*Castor canadensis*) herbivory and dam building (Mouw et al. 2013). The Kwethluk becomes ice-free in May and freezes again in November (Roettiger et al. 2004). The climate is dry, averaging approximately 50 cm/year of precipitation, with most of it falling as rain in late summer. Downstream from the head waters, the mainstem Kwethluk is a low-gradient wandering river with an anastomosing, sometimes anabranching network of primary, secondary, and tertiary channels with many flood channels and lateral habitat types – a shifting habitat mosaic (after Stanford et al. 2005).

### Groundwater sampling methods

The sample was collected in wells. Groundwater wells were installed using a hollow 5.0 cm diameter metal piezometer pounded into the substrate with a centre rod for at least one metre. Then, the centre rod was removed and a 3.8 cm piece of PVC was installed with slits cut into the lower half with a circular saw. Once the PVC was fitted into the hole, the metal piezometer was pulled out leaving only the PVC in the substrate.

During sampling events, we lowered a tube into the PVC and water was pumped with a hand-operated bladder (peristaltic) pump and pumped for 10 minutes through a catch

net (100  $\mu\text{m}$ ) suspended in a 20 litre bucket. Pumping continued for 10 minutes regardless of the clarity of the water. Then, the net was inverted and washed so all organisms were collected into the bucket. Finally, the sediment, detritus, and organisms pumped from the wells were poured into Nalgene bottles and preserved with 95% ethanol. The specimens used for morphological and molecular study were stored in alcohol (95%). The specimens were used for molecular study after they were frozen at  $-20^{\circ}\text{C}$ , in 500  $\mu\text{l}$  of an appropriate buffer.

The morphological and molecular descriptions are based on the type series.

### **Morphological study**

In all, 37 specimens were used for the morphological study: 32 females and five males collected on 24 June 2012 in well #5, gravel bar, Kwethluk River, a tributary of the Kuskokwim River. They constitute the type series of the new species described herein.

A complete dissection of all anatomical parts of all specimens was done and kept as permanent preparations (special metal slides, glycerine gelatine stained with methylene blue as the mounting medium). Anatomical examinations were performed using an oil immersion lens (100 $\times$ ) of a Zeiss interference microscope with a drawing tube. This material was deposited in the Collection of Arthropoda not Insecta of the Museo Nacional de Ciencias Naturales, Madrid (MNCN).

We used the terminology proposed by Serban (1972, and his subsequent papers) for the mandible and male thoracopod VIII. Serban worked in depth on the family Bathynellidae (Serban et al. 1971, Serban 1989a, 1989b), revised and described many genera, and we think that his terminology is the most accurate and intuitive.

### **Specimens collection for DNA extraction**

Five specimens, which are part of the type series, were used for DNA extraction in this study. This type material, the DNA extracted from the five specimens, is deposited in the Tissues and DNA Collection of the Museo Nacional de Ciencias Naturales, Madrid (MNCN). Voucher numbers are shown in Table 2.

To further examine the phylogenetic relationships among bathynellids, we used partial DNA sequences of the mtDNA gene COI (508 base pair, bp). COI sequences were obtained from all individuals.

**Table 2.** Specimens studied in DNA analysis of *Pacificabathynella yupik* sp. nov. from Alaska. Voucher number of specimens of the Tissues and DNA Collection of the MNCN, CSIC (Spain) and GenBank accession numbers.

| Order/Family/Species                     | Habitat               |     | Specimen voucher | GenBank            |
|--|-----------------------|-----|------------------|--------------------|
| Bathynellacea Chappuis, 1915             | Locality              |     | MNCN/ADN         | accessions numbers |
| Bathynellidae Grobden, 1905              | Country               |     |                  |                    |
| <i>Pacificabathynella yupik</i> sp. nov. | Kwethluk River Alaska | USA | 29963            | KP974126           |
| <i>P. yupik</i> sp. nov.                 | Kwethluk River Alaska | USA | 29964            | KP974127           |
| <i>P. yupik</i> sp. nov.                 | Kwethluk River Alaska | USA | 29965            | KP974128           |
| <i>P. yupik</i> sp. nov.                 | Kwethluk River Alaska | USA | 29966            | KP974129           |
| <i>P. yupik</i> sp. nov.                 | Kwethluk River Alaska | USA | 29967            | KP974130           |

### **DNA extraction, amplification, and sequencing**

Whole specimens were placed fully immersed in 0.5 ml digestion buffer (Gilbert et al. 2007), and incubated overnight at 55°C with gentle agitation. The buffer consisted of 5 mM CaCl<sub>2</sub>, 2% sodium dodecyl sulphate (SDS), 40 mM dithiothreitol (DTT), 250 mg/ml proteinase K, 10 mM Tris buffer pH 8, 2.5 mM EDTA (Ethylene-Diamine-Tetra-Acetic acid) pH 8.0, and 10 mM NaCl (final concentrations). After incubating, nucleic acids were extracted from the digestion buffer using a QIAquick PCR purification kit (QIAGEN), (Alda et al. 2007).

A 508 bp region of the COI gene was amplified with the primers C1-J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTCC-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAA AAAATCA-3') (Folmer et al. 1994; Simon et al. 1994). Three microlitres of the DNA solution were used as a template. Other components of the 25 µl PCR reaction were: 1× of the corresponding buffer (75 mM Tris HCl, pH 9.0; 50 mM KCl and 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgCl<sub>2</sub>), 10 mM dNTPs mix, 0.1 µM of both primers, 0.02% BSA, and 0.125 units AmpliTaq Gold® DNA Polymerase (Applied Biosystems). Five microlitres of PCR products were electrophoresed through a 1.5% agarose gel and visualised with SYBR Safe™ DNA Gel Stain (Invitrogen) under ultraviolet light. PCR products were purified by treatment with ExoSAP-IT (USB Amersham, Buckinghamshire, UK) and incubated at 37°C for 45 min, followed by 80°C for 15 min to inactivate the enzyme. Purified PCR product was then used to sequence in both directions using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc., Foster City, USA) in a 10 µl volume, containing 15–20 ng of purified product and 3 pmol of primer. To verify that the sequences obtained came from a bathynellacean, they were compared with sequences from GenBank using Blast (Altschul et al. 1997). The alignment of all bathynellacean COI gene sequences generated in our lab was performed and edited manually using MEGA 5.0 (Tamura et al. 2011). Fine adjustments were made by eye, as the COI does not generally have single gaps. All sequences were submitted to GenBank (see Table 2 for the GenBank Accession Number).

### **Systematic account**

The Family Bathynellidae Grobden, 1905 consists of three subfamilies, Bathynellinae Grobden, 1905, Gallobathynellinae Serban, Coineau et Delamare Deboutteville, 1971 and Austrobathynellinae Delamare Deboutteville and Serban, 1973 and only Bathynellinae is known, until now, from North America.

In 1988, *Pacificabathynella* Schminke and Noodt, 1988 was the only known genus with sexual dimorphism in thoracopod VI of the male. Schminke and Noodt (1988) proposed that the species *Bathynella yezoensis* Uéno, 1954, recorded from wells in Hokkaido (Japan), must belong to the genus *Pacificabathynella*, mainly because of the type of dimorphism, which is similar to the dimorphism seen in *Pacificabathynella sequoiae* Schminke and Noodt, 1988. However, Serban (2000) described a new genus from Japan, *Paradoxibathynella*, which has the same type of sexual dimorphism. He considered, after studying all the characters of *Pacificabathynella yezoensis* (Uéno, 1954), that this species belonged to *Paradoxibathynella* Serban, 2000 and not to *Pacificabathynella* (see Serban 2000 and Camacho, Newell, et al. 2009 for a detailed

discussion). Serban (2000) created the new tribe Pacificabathynellini, which includes the two genera exhibiting sexual dimorphism on thoracopod VI.

The new species described in this paper belongs to the genus *Pacificabathynella* (see Table 1).

Family **BATHYNELLIDAE** Grobben, 1905  
***Pacificabathynella*** Schminke and Noodt, 1988

*Amended genus diagnosis* (after Schminke and Noodt 1988 and Camacho, Newell, et al. 2009) Antennule 7-segmented and antenna 7- or 8-segmented. Mandible with two teeth on incisor process (pars incisiva), processus incisivus accessorius with one tooth and one seta-like tooth, pars molaris with two bidentated structures, parallel to main axis of the teeth, the most distal tooth very strong with apical denticles. Labrum with a small central protuberance at the distal edge. Thoracopod (Th) VI and VIII sexually dimorphic. Male Th VI with 3-segmented endopod, second segment broad and dilated with strong curved seta at outer margin; first segment also broader than usual. Female Th VI with 4-segmented endopod and setal formula 1+0/0+1/0+0/2(1). Male Th VIII with three lobes around genital opening on coxa and with a tooth-like protuberance, prominence in the basipod, exopod and endopod 1-segmented. Female Th VIII bearing epipod; exopod and endopod 1-segmented, both with two or three apical setae. Endopod of uropod with four to eight strong spines along inner margin. Furcal rami with five spines.

Type species: ***Pacificabathynella sequoiae*** Schminke and Noodt, 1988.

Species: ***P. sequoiae*** Schminke and Noodt, 1988.

***P. kalispellensis*** Camacho, Newell, et al., 2009

***P. stanfordi*** Camacho, Newell, et al., 2009

***P. ruthae*** Camacho, Newell, et al., 2009

***P. yupik*** sp. nov.

***Pacificabathynella yupik*** sp. nov.

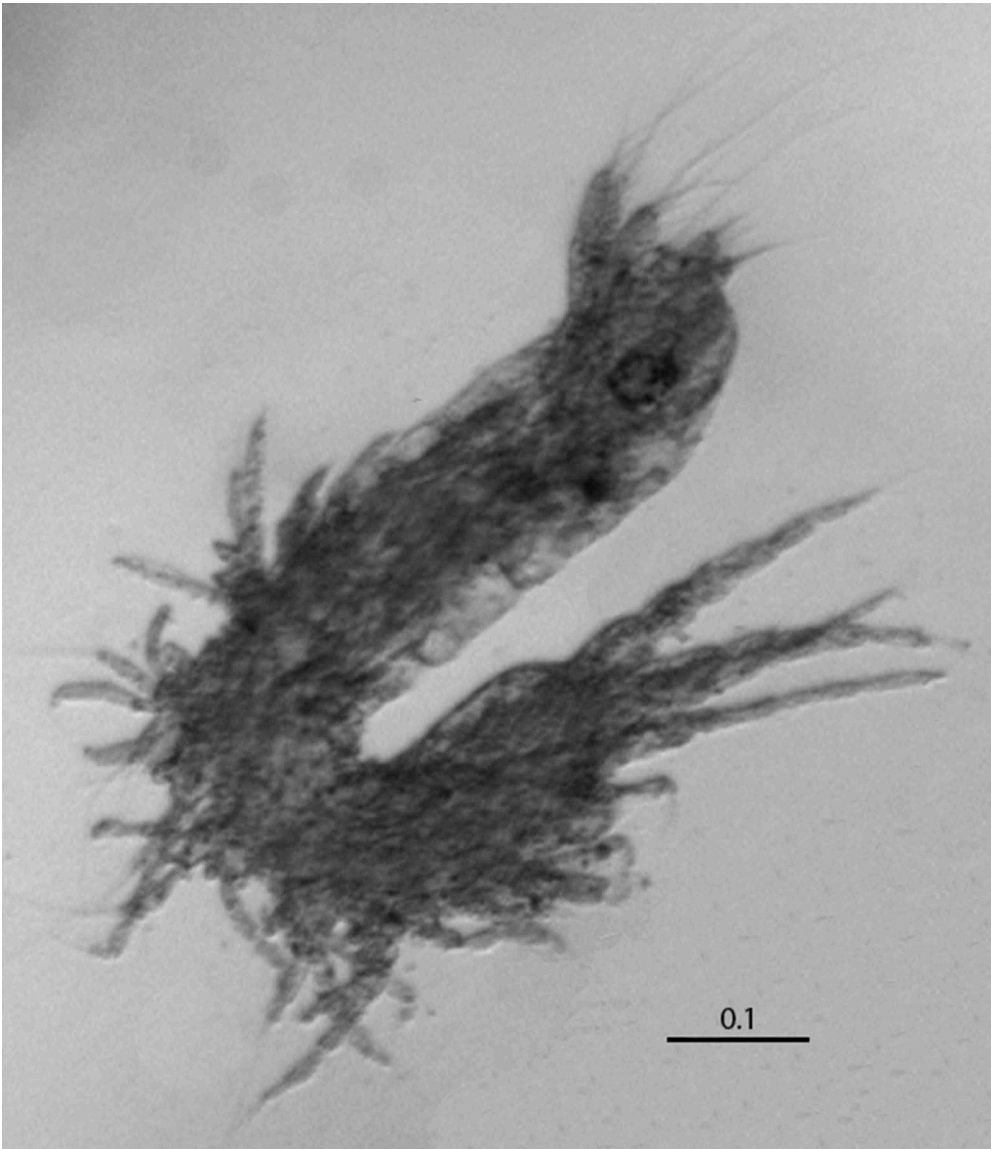
(Figures 1–5)

***Material examined***

***Type locality.*** Kwethluk River, tributary of Kuskokwim River western Alaska (USA); coordinates N60.34520, W161.089146 and Z 1268 (WGS84); 24 June 2012 (32 females, five males and five subadult specimens); collected by Zach Crete. All specimens collected belong to the type series, 37 morphotypes, together with DNA extractions from five subadult specimens used for molecular analysis, five DNA types.

Details of the description are based on all adult specimens. The holotype is a male (MNCN 20.04/10092), the allotype is a female (MNCN 20.04/10093) and the morphological type series contains four males (MNCN 20.04/10125 to 10128) and 31 females (MNCN 20.04/10094 to 10124). The molecular type series contains five DNAtypes (MNCN/ADN 29963 to MNCN/ADN 29967).

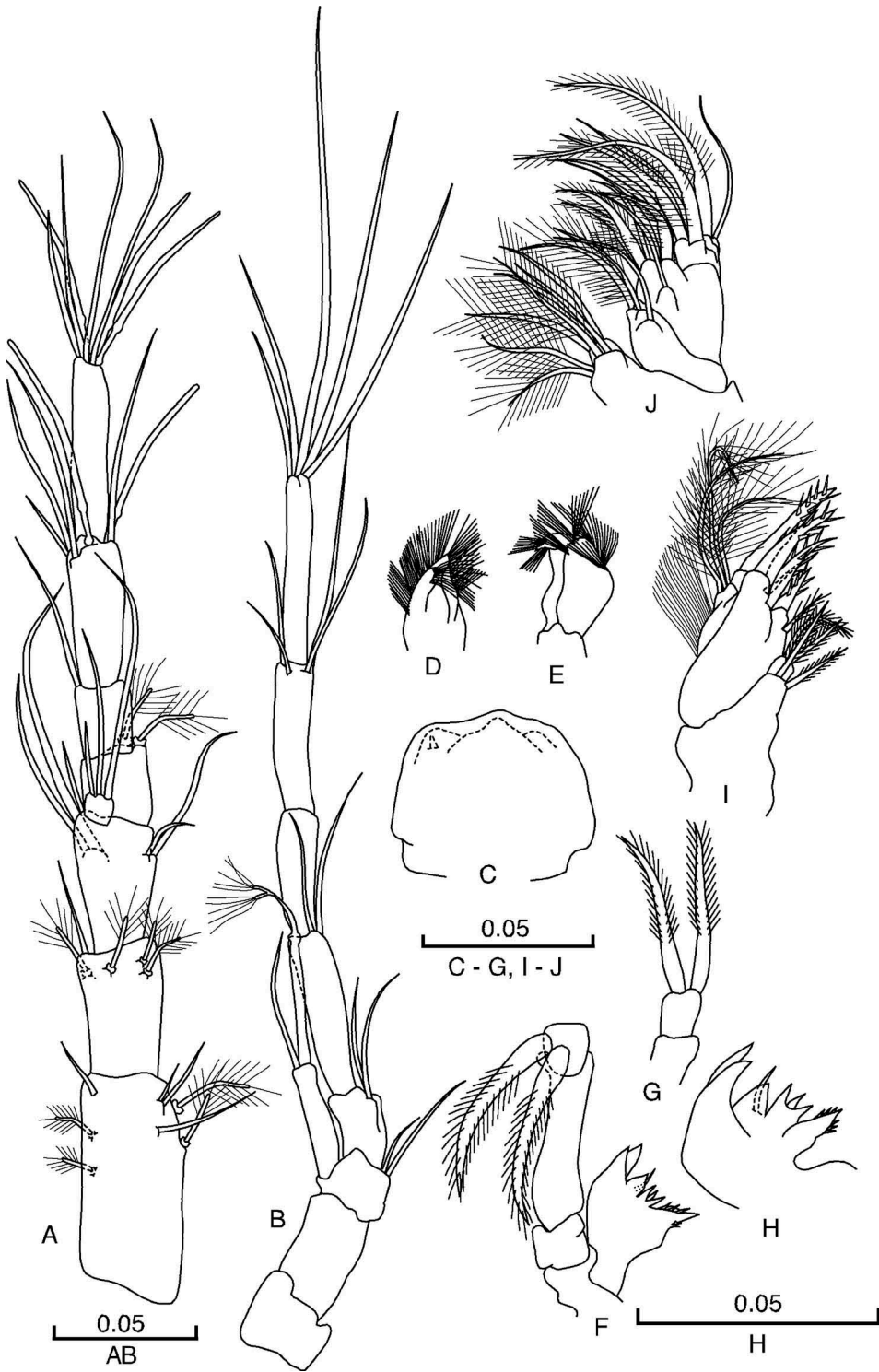
Abbreviations used: Th, thoracopod; A.I, antennule; A.II, antenna; Md, mandible, Mx.I, maxillule and Mx.II maxilla.



**Figure 1.** *Pacificabathynella yupik* sp. nov., male habitus. 10× microscope photography (MNCN).

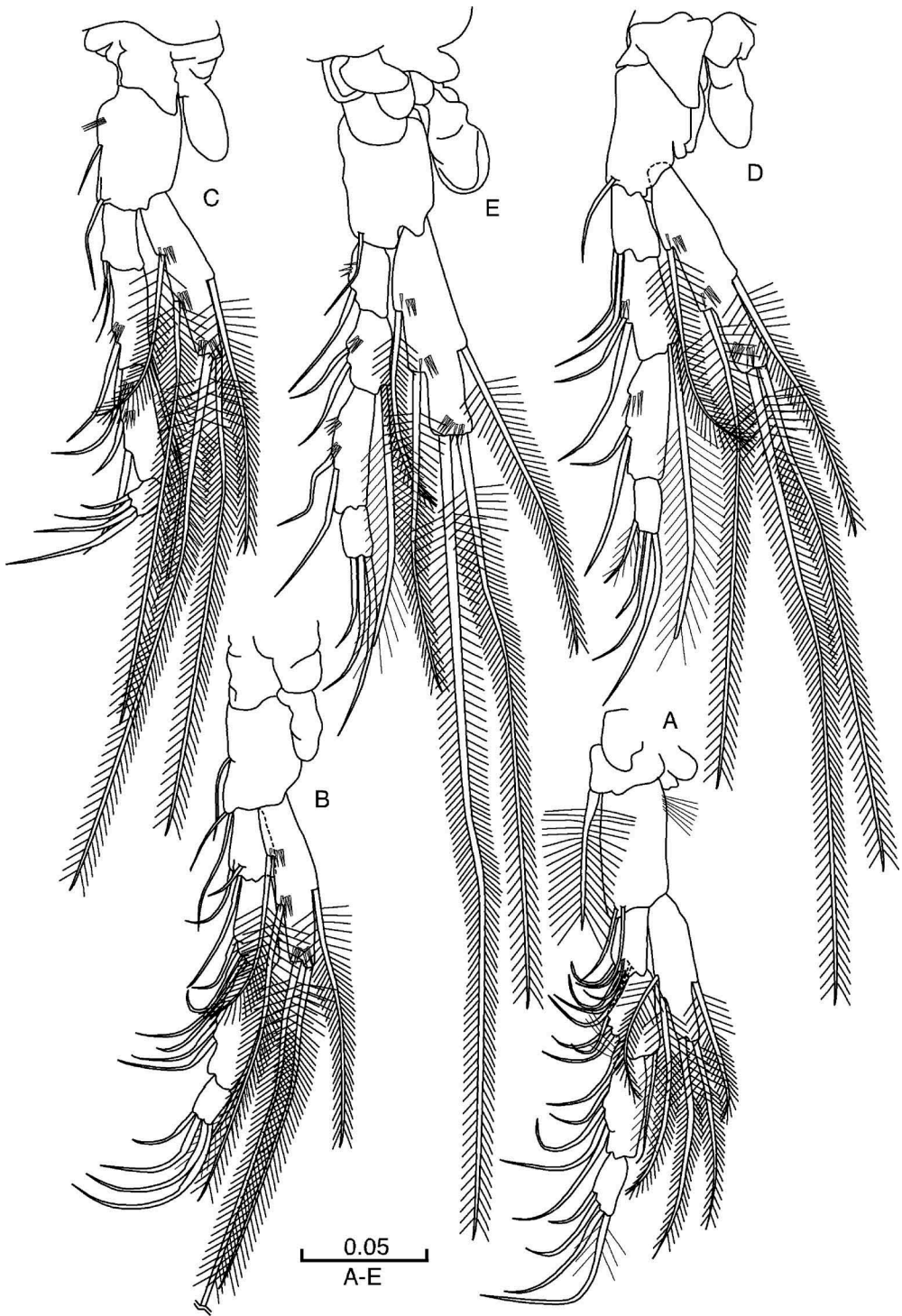
### **Description**

**Body.** Total length of holotype 1.11 mm and allotype 1.11 mm. Total length of males 1.11–0.90 mm, of females 1.18–0.74 mm. For variability see [Table 3](#). Body not very elongated; almost cylindrical, approximately nine times as long as wide; segments slightly widening towards posterior end. Head as long as wide. Pleotelson with one plumose dorsal seta on either side, similar to furcal rami. All drawings are of the holotype (male) except for Th VIII and one figure of Md, paragnath and Th VI that belong to the allotype (female).

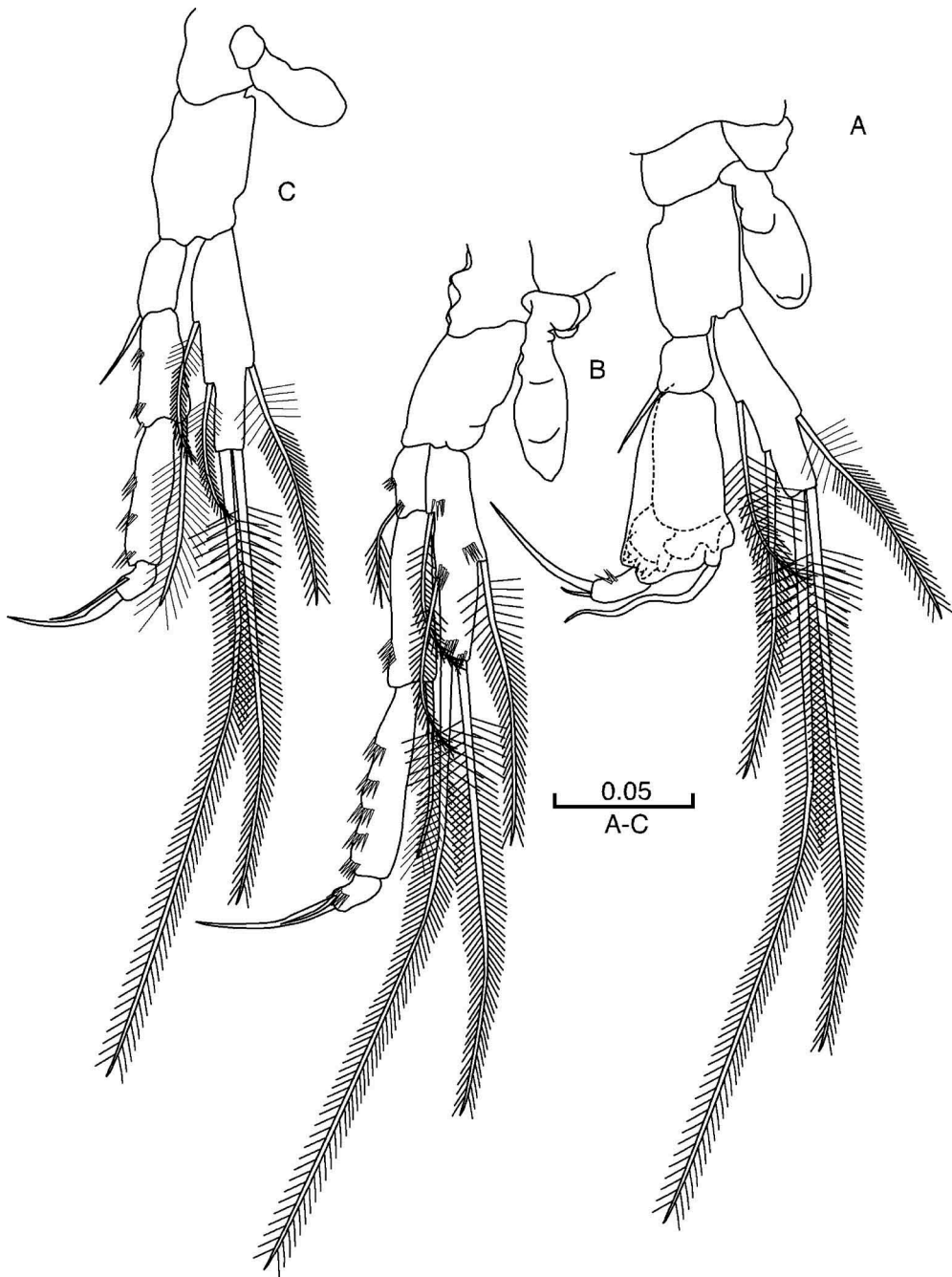


**Figure 2.** *Pacificabathynella yupik* sp. nov., male holotype. (A) Antennule (dorsal view); (B) antenna (dorsal view); (C) Labrum; (D) paragnath; (E) allotype female paragnath; (F) mandible; (G) allotype female palp, mandible; (H) mandible, masticatory part; (I) maxillule and (J) maxilla (dorsal view). Scale bar in mm.



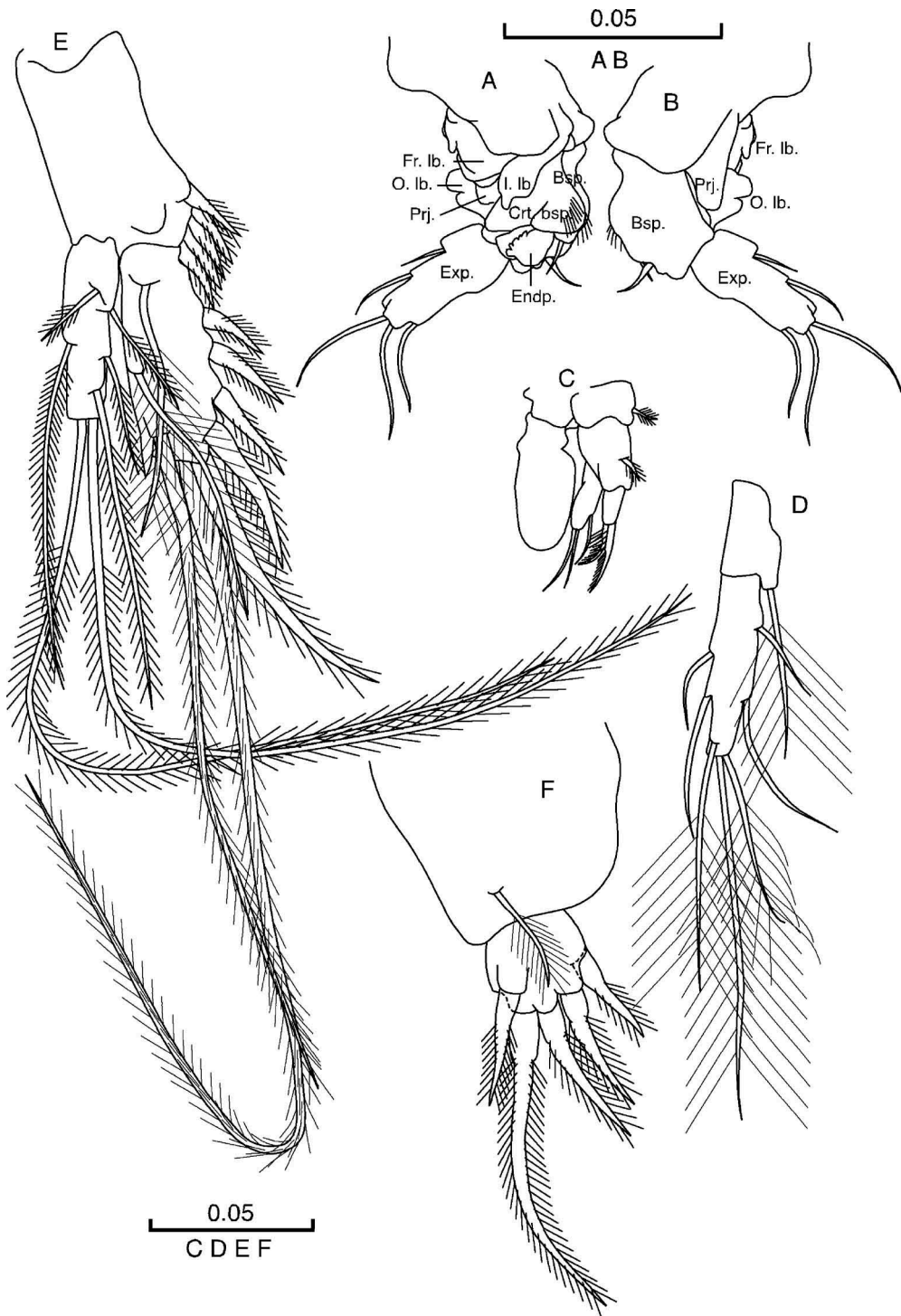


**Figure 3.** *Pacificabathynella yupik* sp. nov., male holotype. (A) Thoracopod I; (B) thoracopod II; (C) thoracopod III; (D) thoracopod IV; (E) thoracopod V. Scale bar in mm.



**Figure 4.** *Pacificabathynella yupik* sp. nov., (A,B) male holotype. (A) Thoracopod VI; (B) thoracopod VII; (C) thoracopod VI female (allotype). Scale bar in mm.

**Antennula (Figure 2A).** Seven-segmented; length of first three segments similar to other four segments combined; segments four and five smaller than others and with similar length; sixth segment shorter than seventh that is the longest; inner flagellum



**Figure 5.** *Pacificabathynella yupik* sp. nov., (A,B, D–F) male holotype. (A) thoracopod VIII (latero-internal view); (B) thoracopod VIII (latero-external view); (C) thoracopod VIII female allotype (frontal view); (D) first pleopod; (E) uropod (latero-external view); (F) furcal rami (dorsal view). Scale bar in mm. Abbreviations: F. lb, frontal lobe; I. lb, inner lobe; O. lb, outer lobe; Prj, projection of inner lobe; Bsp, basipod; Crt. bsp, crest-like protruberance of basipod; Endp, endopod; Exp, exopod.

**Table 3.** Size in mm of the specimens of *Pacificabathynella*, *P. kalispellensis*, *P. stanfordi*, *P. ruthae*, *P. yupik* sp. nov. found in the populations of the different localities of Montana and Alaska (USA). Max = maximum size; Min = minimum size and X = arithmetical mean size.

| Species                  | Sampling point | Date     | Male |      |      | Specimen number | Female |      |      | Specimen number |
|--------------------------|----------------|----------|------|------|------|-----------------|--------|------|------|-----------------|
|                          |                |          | Max. | Min. | X    |                 | Max.   | Min. | X    |                 |
| <i>P. kalispellensis</i> | St-SR Well     | 31/05/89 | 1.01 | 1.01 | 1.01 | 1               | —      | —    | —    | 0               |
|                          | SR Well        | 02/04/89 | 1.42 | 1.31 | 1.36 | 2               | 1.44   | 1.30 | 1.38 | 3               |
|                          | Chris B        |          | 1.06 | 0.95 | 1.00 | 2               | 1.02   | 0.84 | 0.93 | 2               |
| <i>P. stanfordi</i>      | Tadpole B      | 30/04/89 | —    | —    | —    | 0               | 1.46   | 1.10 | 1.34 | 6               |
|                          | St-SR Well     | 31/05/89 | 0.71 | 0.66 | 0.68 | 2               | 0.62   | 0.62 | 0.62 | 1               |
|                          | SR well        | 02/04/89 | 0.72 | 0.66 | 0.69 | 2               | 0.65   | 0.65 | 0.65 | 1               |
|                          | Graham-up      | 14/04/89 | 0.82 | 0.53 | 0.72 | 4               | 0.81   | 0.58 | 0.71 | 5               |
|                          | Channel down   | 01/05/89 | 0.82 | 0.71 | 0.76 | 6               | 0.78   | 0.71 | 0.75 | 6               |
|                          | Walters well   | 31/03/89 | 1.02 | 0.66 | 0.78 | 10              | 0.81   | 0.56 | 0.72 | 6               |
|                          | Graham-west    | 16/07/89 | —    | —    | —    | 0               | 0.84   | 0.67 | 0.75 | 6               |
|                          | Sargent North  | 06/11/03 | —    | —    | —    | 0               | 0.78   | 0.51 | 0.70 | 6               |
|                          | Great Bear     | 09/11/03 | 0.82 | 0.71 | 0.76 | 2               | 0.81   | 0.67 | 0.76 | 5               |
| <i>P. ruthae</i>         | Tadpole B      | 30/04/89 | 0.93 | 0.93 | 0.93 | 1               | 0.94   | 0.84 | 0.90 | 3               |
|                          | Great Bear     | 09/11/03 | 1.74 | 1.74 | 1.74 | 1               | 1.69   | 1.45 | 1.58 | 4               |
|                          | Nyack Aquifer  | -/02/04  | 2.07 | 1.57 | 1.76 | 6               | 1.78   | 1.68 | 1.73 | 2               |
| <i>P. yupik</i> sp. nov. | Kwethluk river | 24/06/12 | 1.11 | 0.90 | 1.02 | 5               | 1.18   | 0.74 | 0.99 | 32              |

almost trapezoidal; setation as in [Figure 2A](#); two aesthetascs on sixth and three on seventh segments. A.I slightly longer than antenna.

**Antenna (Figure 2B).** Eight-segmented; slightly shorter than A.I; first four segments almost as long as fifth and sixth; eighth terminal segments slightly longer than seventh and is the longest; setal formula:  $0/0/2+exp/2+0/2+0/0/2+2/5$ ; exopod, as long as second segment, with two terminal setae, one of these a bifurcated sensory seta; ventromedial seta absent.

**Labrum (Figure 2C).** With a small central protuberance at the distal smooth free edge, flanked by slight protrusions, most evident on ventral side and with a visible small denticle in one of these protrusions.

**Paragnath (Figure 2D, E).** Short, almost square, with rounded distal part and very thick setulation on all surfaces on distal half.

**Mandible (Figure 2F and G).** Palp with three segments, terminal segment ([Figure 2G](#)) with two long and strong barbed claws, more or less cylindrical without expansions. Masticatory part ([Figure 2F, H](#)): incisor process (*pars incisiva*) with two teeth; *processus incisivus accessorius* with one tooth and one long seta-like tooth; *pars molaris* with two dentate structures, like two crowns, parallel to main axis of teeth, the first (the nearest to *processus incisivus accessorius*) with consistently three strong denticles and the other with small denticles, except the most distal which is a strong tooth.

**Maxillule (Figure 2I).** Proximal endite with four setae; distal endite with six teeth, four with denticles and two more, setae-like, and with three plumose setae and tufts of long setules on outer margin.

**Maxilla (Figure 2J).** Four segments; setal formula 7, 3, 7, 5.

**Thoracopods I–VII (Figures 3A–E, 4A–B).** Sexual dimorphism present on Th VI. Th I (Figure 3A) smaller than others; Th II (Figure 3B) to V (Figure 3E) similar in size and Th VII (Figure 4B) a few longer than others. Th I without epipod; coxa with a long and strong plumose seta; basipod with two smooth setae and a tuft of long fine setules near base. Epipod present on Th II–VII, small in Th II–V, half the length of basipod; as long as the basipod on Th VI and VII. Exopod with one segment on all thoracopods, shorter than endopod in all cases, almost like the first two segments in Th I–III and Th VII and slightly longer than the first two segments in Th IV and V; with five barbed setae, two terminal, one dorsal and two ventral. Endopod with four segments in all thoracopods, setal formulae (number of setae on basipod in brackets):

Th I: (2) 5+0/5+1/4+0/4

Th II: (3) 3+0/3+1/3+0/4

Th III: (2) 2+0/2+1/2+0/4

Th IV: (1) 2+0/2+1/2+0/4

Th V: (1) 1+0/2+1/2+0/3

Th VI: (0) 1+0/0+1/2(1)

Th VII: (0) 1+0/0+1/0+0/2(1)

*Thoracopod VI (Figure 5A,B)* with aberrant structure, with only three segments in endopod: first segment broader than usual, second segment broad and dilated bearing on outer margin strong medially curved seta, third segment small with two setae, one long and another shorter.

**Male thoracopod VIII (Figure 5A,B).** Longitudinal axis of coxa and basipod form 25° angle. Penial region with frontal lobe, inner lobe, outer lobe. Frontal lobe with two lobules on distal end. Inner lobe more or less similarly developed as frontal lobe, distal region with projection claw (Prj) on internal side. Outer lobe with two well-developed lobules. Basipod very large, with rows of setules on inner side, with distal and very well-developed, crest-like protuberance and with one lateral-subdistal smooth seta and other distal. Endopod one-segmented, small, 1/4 length of exopod, with small denticles. Exopod well developed, longer than basipod, with five setae.

**Pleopod I (Figure 5D).** Two segments, first segment with one very long plumose seta; second segment with seven setae, the three distal of different length and plumose.

**Female thoracopod VIII of the allotype (Figure 5C).** Coxa with one small, barbed lateral seta; very large epipod, twice as long as basipod; endopod one-segmented, with two apical barbed setae of different length; exopod two times as long as endopod, with three setae, two apical, all smooth, but differing in length.

**Female thoracopod VI of the allotype (Figure 4C).** Exopod one-segmented and with five setae, as in other thoracopods; endopod four-segmented, setal formula 1+0/0+1/0+0/2(1). Number of setae on segments of endopod and basipod of thoracopods differs between female and male. Setal formulae of allotype (number of setae of basipod in brackets):

Th I: (3) 5+0/4+1/4+0/4

Th II: (4) 2+0/4+1/3+0/4

Th III: (2) 3+0/3+1/3+0/4

Th IV: (2) 2+0/2+1/2+0/4

Th V: (1) 2+0/2+1/2+0/4

Th VI: (0) 1+0/0+1/0+0/2(1)

Th VII: (1) 1+0/0+1/0+0/2(1)

**Uropods (Figure 5E).** Sympod almost as long as endopod, rectangular, twice as long as wide, with five distal equal spines; endopod 15% longer than exopod, with five strong claws (distal two longest, the most distal almost twice as long as fourth and almost four times as long as most basal, and the latter slightly shorter than the second), with two very long distal barbed setae and two plumose setae located dorsolaterally; exopod with seven setae, two terminal, three medial and two basal. Endopod with spinous projection at the distal outer corner.

**Pleotelson (Figure 5F).** With one long, plumose dorsal seta on either side near base of furca.

**Furcal rami (Figure 5F).** Almost square, bearing five spines; dorsal spine similar to third, slightly longer than fourth and almost 70% length of second spine and first spine twice as long as second spine.

### Variability

The observed variability affects the number of setae of the different segments of the endopods of the thoracopods I to III on females (see Table 4).

The setal formulae of females different from those of the allotype (number of setae of basipod in brackets):

Th I: (3)5+0/4–5+1/3–4+0/4

Th II: (4) 3+0/3–4+1/3+0/4

Th III: (2) 2–3+0/2–3+1/3+0/4

### Etymology

The species name, as a tribute, is taken from the name Yup'ik Eskimo, the Alaska native people on the lower Kuskokwim where the new species was collected. Substantive in apposition.

### Remarks

*Pacificabathynella yupik* sp. nov. is small within the genus, similar in size to *P. stanfordi* and is as small as *P. kalispellensis* (Table 3). This is the species with the highest number of unique characters within the genus (see Tables 5 and 4): A II eight-segmented and slightly shorter than A I; the pars molaris of the mandible has fewer teeth than in other species; on the second segment of the maxilla has only three setae (Figure 2J), whereas it has four setae in all other congeners; the setal formulae of the endopods of the thoracopods are unique (see Table 4) and are more like *P. stanfordi*, which has the

**Table 4.** Setal formula and other characters of the thoracopods of species of the genus *Pacificabathynella* Schminke and Noodt, 1988, from North America. The number of setae on the basipod appears in brackets. Abbreviations: Th., thoracopod; Endp., endopod; Exp., exopod; F, female; M, male.

|                 | <i>P. sequoiae</i>      | <i>P. kalspellensis</i>    | <i>P. stanfordi</i>     | <i>P. ruthae</i>        | <i>P. yupik</i> sp. nov.  |
|-----------------|-------------------------|----------------------------|-------------------------|-------------------------|---------------------------|
| Th I: endp. M   | (3) 5+0/5+1/4+0/5       | (3) 6-8+0/8-9+1/8-11+0/7-8 | (3) 4-5+0/3-4+1/3-4+0/4 | (3) 6+0/5-6+1/5-6+0/5   | (2) 5+0/5+1/4+0/4         |
| endp. F         | absent                  | (4) 8-10+0/9-10+1/9-11+0/8 | (3) 4-5+0/3-4+1/3-4+0/4 | (3) 5-6+0/5-6+1/5-6+0/5 | (3) 5+0/4-5+1/3-4+0/4     |
| Epipod          | absent                  | 6 setae                    | absent                  | absent                  | absent                    |
| Exp.            | 5 setae                 | (5) 5-6+0/6-7+1/6-8+0/6    | 5 setae                 | 6 setae                 | 5 setae                   |
| Th II: endp. M  | (5) 4 + 0/4 + 1/4 + 0/5 | (8) 7-8+0/7+1/6-8+0/6-7-10 | (2) 2-3+0/2-3+1/2-3+0/4 | (4) 4+0/4+1/4+0/5       | (3) 3+0/3+1/3+0/4         |
| endp. F         | absent                  | present                    | (2) 2-3+0/2-3+1/2-3+0/4 | (5) 4-5+0/4-5+1/3-4+0/5 | (4) 3+0/3-4+1/3+0/4       |
| Epipod          | absent                  | 6 setae                    | absent                  | absent                  | present                   |
| Exp.            | 6 setae                 | (4) 4+0/4+1/3+0/5          | 5 setae                 | 6 setae                 | 5 setae                   |
| Th III: endp. M | (4) 4+0/4+1/3+0/5       | (5) 5-6+0/6-7+1/5-6+0/5-6  | (2) 2+0/2+1/2+0/4       | (2) 2+0/3+1/2-3+0/5     | (2) 2+0/2+1/2+0/4         |
| endp. F         | present                 | (7) 6-7+0/6-8+1/7+0/6-7    | (2) 2+0/2+1/2+0/4       | (4) 3+0/3+1/3+0/5       | (2) 2-3+0/2-3 + 1/2 + 0/4 |
| Epipod          | present                 | present                    | present                 | present                 | present                   |
| Exp.            | 6 setae                 | 6 setae                    | 5 setae                 | 6 setae                 | 5 setae                   |
| Th IV: endp. M  | (2) 3+0/3+1/3+0/4       | (5) 4-5+0/5-6+1/5+0/5      | (1) 2+0/2+1/1-2+0/3     | (3) 2+0/2-3+1/2-3+0/4-5 | (1) 2+0/2+1/2+0/4         |
| endp. F         | present                 | (6) 5-6+0/5-7+1/7+0/6-7    | (1) 2+0/2+1/1-2+0/3     | (4) 3+0/3+1/3+0/5       | (2) 2+0/2+1/2+0/4         |
| Epipod          | present                 | present                    | present                 | present                 | present                   |
| Exp.            | 6 setae                 | 6 setae                    | 5 setae                 | 6 setae                 | 5 setae                   |
| Th V: endp. M   | (1) 2+0/2+1/2+0/3       | (3) 3+0/3+1/4+0/4          | (1) 1+0/1+1/1-2+0/3     | (1) 2+0/2-3+1/2+0/4     | (1) 1+0/2+1/2+0/3         |
| endp. F         | present                 | (6) 3-4+0/4-5+1/4-5+0/5    | (1) 1+0/1+1/1-2+0/3     | (4) 2-3+0/2-3+1/2-3+0/5 | (1) 1+0/2+1/2+0/3         |
| Epipod          | present                 | present                    | present                 | present                 | present                   |
| Exp.            | 6 setae                 | 6 setae                    | 5 setae                 | 6 setae                 | 5 setae                   |
| Th VI: endp. M  | (1) 1+0/0+1/0+0/2(1)    | (0) transformed            | (0) transformed         | (1) transformed         | (0) transformed           |
| endp. F         | (1) 1+0/0+1/0+0/2(1)    | (1) 1+0/0+1/0+0/2(1)       | (1) 1+0/0+1/0+0/2(1)    | (1) 1+0/0+1/0+0/2(1)    | (0) 1+0/0+1/0+0/2(1)      |
| Epipod          | present                 | present                    | present                 | present                 | present                   |
| Expod           | 6 setae                 | 6 setae                    | 5 setae                 | 6 setae                 | 5 setae                   |
| Th VII: endp. M | (1) 1+0/0+1/0+0/2(1)    | (1) 1+0/0+1/0+0/2(1)       | (0) 1+0/0+1/0+0/2(1)    | (1) 1+0/0+1/0+0/2(1)    | (0) 1+0/0+1/0+0/2(1)      |
| endp. F         | (1) 1+0/0+1/0+0/2(1)    | (1) 1+0/0+1/0+0/2(1)       | (0) 1+0/0+1/0+0/2(1)    | (1) 1+0/0+1/0+0/2(1)    | (0) 1+0/0+1/0+0/2(1)      |
| Epipod          | present                 | present                    | present                 | present                 | present                   |
| Exp.            | 6 setae                 | 6 setae                    | 5 setae                 | 6 setae                 | 5 setae                   |



**Table 5.** Characters of the species of the genus *Pacificabathynella* Schminke and Noodt, 1988 found in North America. *P. sequoiæ* Schminke and Noodt, 1988; *P. kalispellensis*; *P. stanfordi*, *P. ruthae* and *P. yupik* sp. nov. Abbreviations: A.I, antennule; A.II, antenna; bsp., basipod; Cr., crest of the basipod; endp., endopod; exp., exopod; segs., segments; DS, dorsal spine of the furca; S1, spine 1 of the furca; S2, spine 2 of the furca; S3, spine 3 of the furca; S5, spine 5 of the furca.

|                            | <i>P. sequoiæ</i> | <i>P. kalispellensis</i> | <i>P. stanfordi</i> | <i>P. ruthae</i> | <i>P. yupik</i> sp. nov. |
|----------------------------|-------------------|--------------------------|---------------------|------------------|--------------------------|
| A.II: setal formula        | 0/2/2/2/0/3/5     | 0/2/3/1/2/6/8/5          | 0/2/2/2/0/4/5       | 0/2/2/2/0/4/5    | 0/0/2/2/2/0/4/5          |
| A.I/A.II                   | A.I<A.II          | A.I<A.II                 | A.I<A.II            | A.I=A.II         | A.I>A.II                 |
| Mx.II: setal formula       | 7/4/7/5           | 7/4/7/6                  | 7/4/7/5             | 7/4/7/5          | 7/3/7/5                  |
| Epipod of Th I             | absent            | absent                   | absent              | absent           | absent                   |
| Epipod of Th II            | absent            | present                  | absent              | absent           | present                  |
| Exopod of Th I             | 5 setae           | 6 setae                  | 5 setae             | 6 setae          | 5 setae                  |
| Exopod of Th II to VII     | 6 setae           | 6 setae                  | 5 setae             | 6 setae          | 5 setae                  |
| Th VIII female             | exp.>>endp.       | exp.>>endp.              | exp.>endp           | exp.>endp        | exp.>>endp.              |
| Size of epipod             | 2 times bsp       | 3 times bsp              | 2 times bsp         | 1.5 times bsp    | 2 times bsp              |
| Setae coxa                 | 2 plumose         | 2 plumose                | 1 plumose           | 1 plumose        | 1 plumose                |
| Seta basipod               | 1 smooth          | 1 plumose                | 1 smooth            | 1 smooth         | 1 plumose                |
| Number setae exp.          | 2 smooth          | 3 smooth                 | 2 smooth            | 2 smooth         | 3 smooth                 |
| Number setae endp.         | 2 smooth          | 2 barbed                 | 2 smooth            | 2 smooth         | 2 barbed                 |
| Th VIII male: Bsp.: setae  | 2                 | 1                        | 1                   | 2                | 2                        |
| Exp.: long/width (setae)   | 3 times (5)       | 2 times (4)              | 2 times (5)         | 3 times (5)      | 2 times (5)              |
| Endopod: setae             | 1 + 1             | 1                        | 1                   | 1                | 0                        |
| Cr. Basipod                | not pronounced    | very pronounced          | very pronounced     | pronounced       | very pronounced          |
| Pleopod: setae             | 1/–               | 1/7                      | 1/6                 | 1/7              | 1/7                      |
| Uropod: Sympod: spines     | 8                 | 8                        | 6                   | 6                | 5                        |
| Endp.: spines              | 4                 | 8                        | 5                   | 6                | 5                        |
| Endp./symp.                | Symp.>endp.       | Symp.<endp.              | Symp.>endp.         | Symp.>endp.      | Symp. = endp             |
| Exp. setae                 | 8                 | 8                        | 7                   | 9                | 7                        |
| Exp./endp.                 | Exp.< endp.       | Exp.< endp.              | Exp.< endp.         | Exp.< endp.      | Exp.< endp               |
| Furca: first spines/second | 3 times longer    | 2.3 times longer         | 1.3 times longer    | 1.5 times longer | 2 times longer           |
| first spines/dorsal        | 7 times longer    | 2 times longer           | 2 times longer      | 3 times longer   | 3 times longer           |
| size spines                | DS<S1>S2=S3>S4    | S1>DS>S2=S3>S4           | S1>DS>S2>S3>S4      | S1>DS>S2=S3>S4   | S1>S2>S3 = DS>S4         |
| Males: Min.-Max. length    | 1.32              | 0.95–1.42                | 0.53–1.02           | 1.57–2.07        | 0.90–1.11                |
| Females: Min.-Max. length  | 1.27              | 0.84–1.46                | 0.51–0.94           | 1.45–1.78        | 0.74–1.18                |



scantiest setal armature, with exopod having only five setae in both species (six in other three spp.); the endopod of male Th VIII has no seta (Figure 5A, B); this species has low number of spines on the endopod of the uropod (five) as *P. stanfordi* and the endopod of the uropod is similar to the sympod in size.

*Pacificabathynella yupik* sp. nov., despite its several unique characters, closely resembles *P. stanfordi* except in body size (see Table 5 and figures). On the uropod, both species have five spines on the endopod and seven setae on the exopod, but differ in their sympodal armature. The furca of the new species is closest to *P. kallispellensis*, the first spine being much longer than the others, but the dorsal spine is shorter in the new species and small in *P. Sequoiae*. All furcal spines are very similar in the other species. Thoracopod VIII in both sexes of the new species is closest to the condition in *P. kallispellensis*.

## Discussion

*Pacificabathynella* and *Paradoxibathynella* are the only known Bathynellidae genera in the world that show sexual dimorphism in the endopod of Th VI. The transformations of Th VI in the male are similar in both genera. Table 1 shows the characteristics of both genera. The new species undoubtedly belongs to the genus *Pacificabathynella* as evidenced by several characters. Thoracopod VIII of the male of the new species has three lobes in the penial region with a protuberance or projection as a claw, whereas *Paradoxibathynella* has four lobes; the basipod has a crest-like protuberance, which is well developed in the new species, but it is lacking in *Paradoxibathynella*. The pars molaris of the mandible has two dentate structures in the new species, as opposed to six teeth in *Paradoxibathynella*. The coxa of the female Th VIII has one plumose seta in the new species, but three setae in *Paradoxibathynella*. The second segment of the first pleopod has seven setae in the new species, whereas there are eight or nine in *Paradoxibathynella*. The sympod of the uropod of the new species has five spines versus four in *Paradoxibathynella*, and five spines on the endopod versus four spines in *Paradoxibathynella*. In general, *Paradoxibathynella* has fewer spines and setae than *Pacificabathynella*.

Tables 5, 3 and 4 show the similarities and differences between the five species of the genus *Pacificabathynella*.

The new species, as we have seen, also shows affinities with *P. kallispellensis*.

The sexual dimorphism is similar in all species and is only exhibited in Th VI and Th VIII. In males there are only three segments on the endopod, with the second segment broadly dilated, bearing on its outer edge a strong and curved medial seta. The first segment is also broader than usual, and the third segment is similar to the fourth segment of the female Th VI and Th VII of the male and female.

## Remarks on the distribution of bathynellaceans in USA

So far, only 17 bathynellids species are known in North America, despite its vastness. Even the first finding (*Texanobathynella bowmani* Delamare DeBoutteville et al., 1975) is relatively recent. To date, the family Parabathynellidae includes only 10 known species, belonging to five genera that inhabit the states of California, Virginia, Texas, and

Montana (*Califobathynella* Cho, 1997, *Californibathynella* Camacho and Serban, 1998, *Hexabathynella* Schminke, 1972, *Texanobathynella* Delamare, Coineau et Serban, 1975 and *Montanabathynella* Camacho, Stanford, et al., 2009). There are large areas to be sampled so presumably there are many new species that remain to be discovered. Until 2000 only four species of the family Bathynellidae were known, in two genera, *Bathynella* and *Pacificabathynella* from Colorado and California, respectively. We described in 2009 three species found in the state of Montana, extending the range of distribution of *Pacificabathynella* (see Camacho, Newell, et al. 2009) by over 1700 km. Now, the new species from Alaska widens the distribution range for this family 3000 km further north.

Many species found by different workers have not yet been formally described: Noodt (1974) reported bathynellids from California; Pennak and Ward (1985) reported bathynellids having been collected by their colleagues in the states of Montana, Wyoming, Colorado, Kansas, Oklahoma, Indiana, Ohio, and Georgia. Newell and Camacho have studied various populations of Montana, Texas, and Washington State, which have not yet been formally described, but some of them do belong to *Pacificabathynella*.

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