**Sisubiotus hakaiensis** sp. nov. (Tardigrada, Macrobiotidae), a new tardigrade species from Calvert Island (British Columbia, Canada)

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**Abstract.** Tardigrades reports from British Columbia (Canada) trace back to 1908 and numerous species have been recorded from this region, despite the relatively few published sampling studies. We describe by integrative taxonomy (light microscopy morphology, morphometrics, and DNA sequencing) a new tardigrade species, *Sisubiotus hakaiensis* sp. nov. from the British Columbia central coast. The new species has been found in moss collected from a vertical rock outcrop near the Hakai Institute Calvert Island Field Station. *Sisubiotus hakaiensis* sp. nov. differs from all the other known species in the genus by the presence of a labyrinthine layer inside the egg process walls, whereas no consistent differences in the animals were found. This unique egg characteristic therefore required the amendment of the *Sisubiotus* generic diagnosis to account for the presence of the labyrinthine layer inside the egg process walls.

**Keywords.** Hakai, BC central coast, egg morphology, tardigrades, integrative taxonomy.


**Introduction**

Reports of tardigrades from Canada can be traced back to two publications in the early 20th century: Richters (1908), who identified three species collected from Vancouver, British Columbia during a Pacific expedition from 1896–1897; and Murray (1910), who collected 31 species during his limited Canadian survey. To date, approximately 121 species of tardigrades are known in Canada, and almost half of these (58 species from 32 genera) were recorded from British Columbia (Meyer 2013; Kaczmarek...
et al. 2016). Of 58 species known from British Columbia, six have their type locality in the province: *Echiniscus canadensis* Murray, 1910; *Echiniscus reymondi* Marcus, 1928; *Insuetifurca arrowsmithi* (Kathman & Nelson, 1989); *Ursulinius woodsae* (Kathman, 1990); *Macrobiotus occidentalis* Murray, 1910; and *Platicrissa cheuleensis* Kathman, 1990. The genus *Sisubiotus* was recently erected (Stec et al. 2021a) based on both morphological and molecular data to accommodate for three species previously assigned to *Macrobiotus* C.A.S. Schultze, 1834. This genus can be distinguished from the otherwise similar *Macrobiotus* by the absence of cuticular pores, the presence of elongated teeth in the second band of the oral cavity armature (OCA), and by a characteristic egg morphology (laid free with areolation and conical processes without the labyrinthine layer) (Stec et al. 2021a). The first two described species, *S. spectabilis* (Thulin, 1928) and *S. grandis* (Richters, 1911), were considered for many years as species inquirenda (Dastych 1973). Maucci & Pilato (1974) showed that these two species are valid, and they can be discriminated by their morphology. *Sisubiotus wuyishanensis* (Zhang & Sun, 2014), described from China (Zhang & Sun 2014) was deemed to be insufficiently characterized to be differentiated from *S. spectabilis* and *S. grandis* and thus considered as species inquirenda by Stec et al. (2021a). Integrative taxonomy integrates multiple lines of evidence from multiple technique (morphological, molecular) to solve taxonomic issues, and it has been providing to be extremely useful in tardigrades taxonomy (see for example Kiosya et al. 2021; Stec et al. 2021b; Stec & Morek 2022). Thus, using an integrative taxonomy approach we here describe *Sisubiotus hakaiensis* sp. nov. from Calvert Island (British Columbia, Canada), which can be easily differentiated from the other three species in the genus by the presence of a labyrinthine layer in the egg processes. Therefore, we also provide an amended diagnosis of the genus considering this new character state for *Sisubiotus*.

**Material and methods**

**Samples and specimens**

Specimens were recovered from moss samples collected by one of the authors (HC) from a vertical rock outcrop near the Hakai Institute Calvert Island Field Station during a biodiversity survey in 2018 and on a subsequent collecting trip by Hakai researchers in 2021. The Field Station is located within the Hakai Lúxvbálís Conservancy of the Province of British Columbia on the central coast of British Columbia. The central coast of British Columbia extends from approximately the north end of Aristazabal Island at ~52°49′ N to the entrance to Queen Charlotte Strait at ~50°59′ N, excluding the offshore Haida Gwaii Archipelago (Lindstrom et al. 2021).

Details on samples and specimens found are shown in Table 1. The samples were examined for tardigrades using the protocol by Stec et al. (2015). Animals and eggs were split into several groups for specific analyses, i.e., morphological analysis in PCM (Phase Contrast Microscopy) and DNA sequencing (for details see Table 1).

**Microscopy and imaging**

Specimens for light microscopy were mounted on microscope slides in a small drop of Hoyer’s (~200 mg) medium, secured with a cover slip (22*22 mm) and dried at 60°C for a week. Freshly mounted specimens were checked for the presence of sperm in the gonad (Coughlan & Stec 2019; Coughlan et al. 2019). Slides were examined under an Olympus BX53 light microscope with PCM, associated with an Olympus DP74 digital camera. All figures were assembled in Figure J (Mutteter & Zinck 2013). For structures that could not be satisfactorily focused on a single light microscope photograph, a stack of 2–3 images were taken with an equidistance of ca 0.2 μm and assembled manually into a single deep-focus image in GIMP ver. 2-10 (GIMP Development Team 2019). Deep-focus images obtained by stacking are indicated in the figures caption with an asterisk (*).
Table 1. Samples details and number of specimens analyzed [animals + eggs].

<table>
<thead>
<tr>
<th>Sample and field numbers</th>
<th>Locality</th>
<th>Coordinates for all samples listed</th>
<th>Elevation m a.s.l.</th>
<th>Coll. date</th>
<th>Sample collector</th>
<th>Specimens analyzed [A+E]</th>
</tr>
</thead>
<tbody>
<tr>
<td>S418 (HC2018-14-1)</td>
<td>Steep wall, trail to Lookout, Calvert Island</td>
<td>51°38′54″ N, 128°8′38″ W</td>
<td>50</td>
<td>15/06/2018</td>
<td>Henry Choong</td>
<td>PCM Paratypes [2 + 2]</td>
</tr>
<tr>
<td>S1910 (GSB-Loc.4)</td>
<td>40</td>
<td>29/06/2021</td>
<td>Gillian Sadlier-Brown</td>
<td>PCM Paratypes [0 + 1]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1911 (GSB-Loc.5)</td>
<td>40</td>
<td>29/06/2021</td>
<td>Gillian Sadlier-Brown</td>
<td>PCM Holotype [1 + 0] +</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PCM Paratypes [52 + 0]</td>
<td>DNA [3 + 0]</td>
</tr>
</tbody>
</table>
**Morphometrics and morphological nomenclature**

All measurements are given in micrometers (μm). Sample size was adjusted following the recommendations by Stec *et al.* (2016). Structures were measured only if their orientation was suitable. Body length was measured from the anterior extremity to the posterior end of the body, excluding the hind legs. Buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). The *pt* index is the ratio of the length of a given structure to the length of the buccal tube (Pilato 1981). Measurements of buccal tube widths, heights of claws and eggs, as well as the terminology used to describe the OCA and eggshell morphology follow Kaczmarek & Michalczyk (2017). Morphometric data were handled using the “Parachela” ver. 1.7 template available from the Tardigrada Register (Michalczyk & Kaczmarek 2013). The raw morphometric data are provided as Supplementary file 1 (SM.01). Tardigrade taxonomy follows Bertolani *et al.* (2014) and Stec *et al.* (2021a).

**Genotyping**

DNA was extracted from individual animals following a Chelex® 100 resin (BioRad) extraction method by Casquet *et al.* (2012) with modifications described in detail in Stec *et al.* (2020a). We sequenced four DNA fragments, three nuclear (18S rRNA, 28S rRNA, ITS2) and one mitochondrial (COI). All fragments were amplified and sequenced according to the protocols described in Stec *et al.* (2020a); primers with original references are listed in Appendix 1. Sequencing products were read with an ABI 3130xl sequencer at the Department of Biological and Environmental Sciences (University of Jyväskylä, Finland).

**Phylogenetic analysis**

The phylogenetic analyses were conducted using concatenated 18S rRNA+28S rRNA+ITS-2+COI sequences. All *Sisubiotus* sequences available in GenBank were used. In addition, representative sequences of Macrobiotidae Thulin, 1928, Adorybiotidae Vecchi, Vecchi & Michalczyk, 2020, Richtersiusidae Guidetti, Schill, Giovannini, Massa, Goldoni, Ebel, Förschner, Rebecchi & Cesari, 2021 and Murrayidae Guidetti, Rebecchi & Bertolani, 2000 were included as outgroups (Appendix 2). Alignment of 18S and 28S markers was done on reference alignments as in Vecchi *et al.* (2022b). The ITS-2 sequences were aligned using MAFFT ver. 7 (Katoh *et al.* 2002; Katoh & Toh 2008) with the G-INS-i method (thread=4, threadtb=5, threadit=0, reorder, adjust direction, any symbol, max iterate=1000, retree=1, global pair input). The COI sequences were aligned according to their amino acid sequences (translated using the invertebrate mitochondrial code) with the MUSCLE algorithm (Edgar 2004) in MEGA7 with default settings (all gap penalties=0, max iterations=8, clustering method=UPGMB, lambda=24). Alignments were visually inspected and trimmed in MEGA7. Sequences were concatenated with the R package ‘concatipede’ ver. 1.0.0 (Vecchi & Bruneaux 2021). Model selection was performed for each alignment partition (6 in total: 18S rRNA, 28S rRNA, ITS-2 and three COI codons) with PartitionFinder2 (Lanfear *et al.* 2016), partitions and models selection process and results are present in Supplementary file 2 (SM.02). Bayesian Inference (BI) phylogenetic reconstruction was done with MrBayes ver. 3.2.6 (Ronquist *et al.* 2012). Two runs with one cold chain and three heated chains were run for 20 million generations with a burning of 2 million generations, sampling a tree every 1000 generations. Posterior distribution sanity was checked with the Tracer ver. 1.7 (Rambaut *et al.* 2018). MrBayes input file with the input alignment is available as Supplementary file 3 (SM.03). The phylogenetic tree was visualized with FigTree ver. 1.4.4 (Rambaut 2007) and the image was edited with Inkscape 0.92.3 (Bah 2011). The complete phylogenetic tree is available in Supplementary file 4 (SM.04).

**Results**

**Phylogenetic analysis**

The phylogenetic analysis (Fig. 1) of the sequences from three individuals confirmed the generic assignation to *Sisubiotus* of the new species. The new species forms a separate clade in sister group.
relationship with (S. spectabilis + S. grandis). The genera Sisubiotus, Macrobiotus, Mesobiotus Vecchi, Cesari, Bertolani, Jónsson, Rebecchi & Guidetti, 2016, Tenuibiotus Pilato & Lisi, 2011 and Paramacrobiotus Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009 are recovered as monophyletic, whereas the genus Minibiotus is paraphyletic (SM.04).

Tardigrade taxa found

Other than the new species of Sisubiotus, the analysed samples contained:

Sample S418: Adorybiotus cf. granulatus.


Sample S1911: Acanthechiniscus goedeni (Grigarick, Mihelčič & Schuster, 1964), Diploechiniscus sp., Hypechiniscus gladiator (Murray, 1905), Calohypsibius ornatus (Richters, 1900), Platicrista sp., Adorybiotus cf. granulatus.

Fig. 1. Bayesian phylogenetic placement of the new species of Sisubiotus Stec, Vecchi, Calhim & Michalczyk, 2021. Values above/below branches are Bayesian posterior probability values. Scale bar indicates mutations/site.
**Systematic and taxonomic account**

Phylum Tardigrada Doyère, 1840  
Class Eutardigrada Richters, 1926  
Superfamily Macrobiotoidea Thulin, 1928  
Family Macrobiotidae Thulin, 1928

*Genus Sisubiotus* Stec, Vecchi, Calhim & Michalczyk, 2021

**Amended diagnosis**

Large and whitish Macrobiotidae with: (i) a poreless cuticle, (ii) a wide rigid buccal tube with well-developed oral cavity armature (all three bands of teeth clearly visible in PCM, anterior teeth of the second band longitudinally elongated), (iii) two macroplacoids and a large microplacoid positioned close to them in the pharynx, (iv) Y-shaped claws of the Macrobiotus type with lunules on each leg, (v) ornamented eggs, laid freely, with areolation and conical processes with or without the labyrinthine layer.

**Genus composition**


*Sisubiotus hakaiensis* sp. nov.  
Figs 2–5, Tables 2–3

**Differential diagnosis**

Reliable differences in differentiating the animal morphology between different species of *Sisubiotus* were not found. By the presence of a labyrinthine layer in the egg processes walls, *S. hakaiensis* sp. nov. can be easily differentiated from *S. spectabilis*, *S. grandis* and *S. wuyishanensis* (labyrinthine layer absent in these three species). In addition, *S. hakaiensis* differs from *S. wuyishanensis* by the presence of granulation on legs (present in *S. hakaiensis* vs absent in *S. wuyishanensis*) and by the shape of the egg processes walls (straight to slightly sigmoidal in *S. hakaiensis* vs concave in *S. wuyishanensis*).

**Etymology**

This species name refers to the Hakai Institute, which conducts and advances long-term scientific research at remote locations at the coastal margin of British Columbia, Canada, and which includes the Calvert Island Field Station from where the samples were collected.

**Material examined**

58 animals and 3 embryonated eggs. Specimens mounted on microscope slides in Hoyer’s medium (55 animals + 3 embryonated eggs) and processed for DNA sequencing (3 animals).

**Holotype**

CANADA – British Columbia • Lookout, Calvert Island, British Columbia; 51°38′54″ N, 128°8′38″ W; 40 m a.s.l.; 29 Jun. 2021; Gillian Sadlier-Brown leg.; moss on rock; JYUt.S1911_SL5_B.

**Paratypes**

CANADA – British Columbia • 35 animals; Lookout, Calvert Island, British Columbia; 51°38′54″ N, 128°8′38″ W, 40–50 m a.s.l.; 15 Jun. 2018, 29 Jun. 2021; Henry Choong & Gillian Sadlier-Brown leg.; moss on rock; JYVt.S418_SL2, JYVt.S1911_SL1 to SL6 • 19 animals; same collection data as for
Voucher specimens are deposited in the Natural history collections of the Jyväskylä University Museum, Ihantolantie 5, Jyväskylä, Finland (JYV), Survontie 9, 40520 Jyväskylä, Finland (Slides JYVt.S1911_SL5, JYVt.S418_SL1-2, JYVt.S1911_SL1 to SL6) and in the Invertebrate Zoology Department, Royal BC Museum (RBCM), 675 Belleville Street, Victoria, BC, Canada (RBCM.S1910_SL2 (RBCM 022-00001-001), RBCM.S1911_SL9, SL10 (RBCM 022-00001-002)).

Description

Animals (measurements and statistics in Table 2)

Body whitish; after fixation in Hoyer’s medium body transparent (Fig. 2A). Eyes present in animals before and after fixation in Hoyer’s medium. Cuticle poreless. Patches of fine granulation on the internal and external surfaces of legs I–III (Fig. 2B–C) as well as dorsal and dorso lateral of legs IV clearly visible in PCM (Fig. 2D). A pulvinus is present on the internal surface of legs I–III (Fig. 2B). Claws slender, of the *hufelandi* type. Primary branches with distinct accessory points, a long common tract, and with an evident stalk connecting the claw to the lunula (Fig. 3). All lunulae smooth (Fig. 3A, D). Single cuticular bar on legs I–III often visible in PCM (Fig. 3C), whereas the horseshoe-shaped structure under

![Image](https://example.com/image.png)
Table 2. Measurements (in μm) of selected morphological structures of the animals of *Sisubiotus hakaiensis* sp. nov. mounted in Hoyer’s medium. Abbreviations: N = number of eggs/structures measured; Range = the smallest and the largest structure among all measured specimens; SD = standard deviation.

<table>
<thead>
<tr>
<th>Character</th>
<th>N</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>Holotype</th>
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<tr>
<td></td>
<td></td>
<td>μm pt</td>
<td>μm pt</td>
<td>μm pt</td>
<td>pt</td>
</tr>
<tr>
<td>Body length</td>
<td>25</td>
<td>387–685</td>
<td>707–1070</td>
<td>573</td>
<td>948</td>
</tr>
<tr>
<td>Buccopharyngeal tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buccal tube length</td>
<td>30</td>
<td>35.7–73.3</td>
<td>–</td>
<td>58.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Stylet support insertion point</td>
<td>30</td>
<td>30.1–61.7</td>
<td>78.9–85.4</td>
<td>48.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Buccal tube external width</td>
<td>25</td>
<td>5.7–14.4</td>
<td>15.2–21.2</td>
<td>10.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Buccal tube internal width</td>
<td>25</td>
<td>3.7–11.5</td>
<td>10.3–17.7</td>
<td>8.3</td>
<td>1.7</td>
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<tr>
<td>Ventral lamina length</td>
<td>25</td>
<td>25.5–51.7</td>
<td>65.2–75.1</td>
<td>40.9</td>
<td>6.2</td>
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<tr>
<td>Placoid lengths</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macroplacoid 1</td>
<td>30</td>
<td>7.1–21.0</td>
<td>17.7–36.4</td>
<td>16.0</td>
<td>3.5</td>
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<tr>
<td>Macroplacoid 2</td>
<td>30</td>
<td>4.5–15.5</td>
<td>12.7–21.2</td>
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<td>2.2</td>
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<tr>
<td>Microplacoid</td>
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<td>7.3–11.4</td>
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<tr>
<td>Macroplacoid row</td>
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<td>34.6–57.2</td>
<td>29.0</td>
<td>6.0</td>
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<tr>
<td>Placoid row</td>
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<td>46.0–72.5</td>
<td>36.9</td>
<td>7.2</td>
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<tr>
<td>Claw 1 heights</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>External primary branch</td>
<td>19</td>
<td>7.4–15.5</td>
<td>19.4–23.2</td>
<td>12.8</td>
<td>1.7</td>
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<tr>
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<td>19</td>
<td>5.4–12.0</td>
<td>14.9–18.0</td>
<td>10.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Internal primary branch</td>
<td>18</td>
<td>9.6–13.7</td>
<td>17.1–21.9</td>
<td>12.3</td>
<td>1.1</td>
</tr>
<tr>
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<td>17</td>
<td>8.4–10.9</td>
<td>12.6–17.6</td>
<td>9.8</td>
<td>0.7</td>
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<td>Claw 2 heights</td>
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<td>19.2–24.2</td>
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<td>12.5–18.9</td>
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<td>1.8</td>
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<tr>
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<td>8.8–11.3</td>
<td>14.5–17.9</td>
<td>9.9</td>
<td>0.7</td>
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<tr>
<td>Claw 3 heights</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>External primary branch</td>
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<td>8.1–15.2</td>
<td>18.9–23.9</td>
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<td>1.6</td>
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<tr>
<td>External secondary branch</td>
<td>16</td>
<td>6.7–12.6</td>
<td>15.5–18.7</td>
<td>10.3</td>
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<tr>
<td>Internal primary branch</td>
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<td>7.6–14.6</td>
<td>17.8–23.1</td>
<td>12.6</td>
<td>1.6</td>
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<tr>
<td>Internal secondary branch</td>
<td>18</td>
<td>5.3–12.0</td>
<td>13.5–18.8</td>
<td>9.9</td>
<td>1.4</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Anterior primary branch</td>
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<td>9.8–20.1</td>
<td>22.0–37.3</td>
<td>17.1</td>
<td>2.4</td>
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<td>6.3–14.5</td>
<td>15.7–26.8</td>
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<td>1.9</td>
</tr>
<tr>
<td>Posterior primary branch</td>
<td>20</td>
<td>8.8–23.6</td>
<td>21.5–43.1</td>
<td>16.1</td>
<td>2.8</td>
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<tr>
<td>Posterior secondary branch</td>
<td>18</td>
<td>6.1–13.8</td>
<td>14.6–23.0</td>
<td>11.3</td>
<td>1.8</td>
</tr>
</tbody>
</table>
claws IV poorly visible only in PCM (Fig. 3D). Mouth antero-ventral. Bucco-pharyngeal apparatus of the Macrobiotus type (Fig. 4A), with the ventral lamina and ten peribuccal lamellae. Pharyngeal bulb spherical, with trapezoidal apophyses with a median constriction, two rod-shaped macroplacoids and a large microplacoid positioned close to them; Fig. 4B–C). The macroplacoid length sequence is 2<1. The first macroplacoid is anteriorly narrowed and constricted in the middle whereas the second has a sub-terminal constriction (Fig. 4B–C). The oral cavity armature well developed and composed of three bands of teeth, always clearly visible under PCM (Fig. 4D–E). The first band of teeth is composed of numerous small teeth visible in PCM as granules (Fig. 4D–E), arranged in several rows, situated anteriorly in the oral cavity, which start behind the bases of the peribuccal lamellae and extend on the lamellae bases. The second band of teeth is situated between the ring fold and the third band of teeth and comprised of 3–4 rows of teeth visible in PCM as granules (Fig. 4D–E) larger than those in the first band. The most anterior row of teeth within the second band comprises larger and longitudinally elongated teeth than the subsequent posterior rows (Fig. 4D–E). The teeth of the third band are located within the posterior portion of the oral cavity, between the second band of teeth and the buccal tube anterior ending (Fig. 4D–E). The third band of teeth is divided into the dorsal and the ventral portion. Under PCM, both bands are divided into three distinct transverse ridges, with the medio-dorsal larger than the medio-ventral one. In some specimens, additional mucrones can occur behind the medio-ventral ridge (Fig. 4E). Typically-shaped stylet furca, with spherical condyles supported by short branches provided with small apophyses (Fig. 4A).

Fig. 3. Sisubiotus hakaiensis sp. nov., claws in PCM. A*, C. Paratype (JYUt.S1911_SL3_A). Claws II–III. B, D. Holotype (JYUt.S1911_SL5_B). Claws IV. Arrowhead indicates horseshoe-shaped structure under claws IV. Deep-focus images obtained by stacking are indicated in the figures caption with an asterisk (*). Scale bars =10 μm.
Fig. 4. *Sisubiotus hakaiensis* sp. nov., buccal-pharyngeal apparatus in PCM. A, D–E. Paratype (JYUt. S1911_SL4_C). B–C. Paratype (JYUt.S1911_SL2_B). A. In toto buccal-pharyngeal apparatus. B–C. Placoids, arrowheads indicate constrictions in the macroplacoids. D*. Dorsal Oral Cavity Armature (OCA), empty arrowheads indicate the three bands of the OCA. E*. OCA, empty arrowheads indicate the three bands of the OCA. Deep-focus images obtained by stacking are indicated in the figures caption with an asterisk (*). Scale bars: A = 50 μm; B–E = 20 μm.
Eggs (measurements and statistics in Table 3)
Laid freely, white, spherical with large conical processes, areolated (Fig. 5A). About ten processes on the circumference. Each process is surrounded by usually eight to twelve deep areolae. Usually, two rows of areolae are present between the neighboring processes (Fig. 5A). The areolae rims are thin and high, and the areolae surface is reticulated (Fig. 5B). The labyrinthine layer between the process walls present and composed by a very fine mesh (Fig. 5C–D). Processes walls straight to slightly sigmoidal, and processes tips usually blunted or flat (Fig. 5C–D).

Fig. 5. Sisubiotus hakaiensis sp. nov. Paratype (JYUt.S418 SL1 A). Eggs in PCM. A. Egg surface. B. Reticulated chorion between areolations. C. Egg process with labyrinthine layer (arrowhead); seen as reticulation. D. Egg process in section showing labyrinthine layer (arrowhead). Scale bars: A = 50 μm; B = 5 μm; C = 20 μm; D = 10 μm.
Reproduction
The examination of animals freshly mounted in Hoyer’s medium revealed the presence of testis filled with sperm, so this species can be considered gonochoric.

DNA sequences
Sequences from 3 individuals from sample S1911 were obtained. 18S rRNA (3 sequences: OM523054-6); 28S rRNA (3 sequences: OM523059-61); COI (2 sequences: OM523181-2); ITS2 (2 sequences: OM523057-8).

Discussion
The analyzed animals and eggs belong to a new species in the genus Sisubiotus, morphologically and phylogenetically distinct from all other species in the genus. The finding of three embryonated eggs allowed us to link the animal and eggs morphologies. The presence of a labyrinthine layer in the egg processes walls is a new character state for the genus, requiring an amendment of its morphological diagnosis. This newly described species increases the number of tardigrade species recorded from British Columbia to 59, of which seven have their type locality specifically in this province. This new species finding highlights how important is to keep sampling in already explored areas as more biodiversity still awaits to be found (see for example Vuori et al. 2020).

The genus Sisubiotus has been reported mostly from Europe (Croatia, Finland, France, Italy, Norway, Poland, Romania, Switzerland), the Arctic (Franz Josef Land) and Asia (China, Japan, Siberia-Russia) (McInnes 1994; Stec et al. 2021a). Only a few records are known for the Americas: S. grandis was found in Greenland and Alaska (Grøngaard et al. 1990; Johansson et al. 2013) whereas S. spectabilis was recorded for Greenland (Maucci 1996), Alaska (USA) (Dastych 1982; Johansson et al. 2013), West Virginia (USA) (Tarter & Nelson 1993) and Argentina (Claps & Rossi 1984). The current description of S. hakaiensis sp. nov. is a valuable addition to the scarce records of this genus in the Americas. It is important to note that reticulation in the egg process is a unique feature in an already uncommon and peculiar genus. Thus, it is unlikely to have been overlooked in previous records of Sisubiotus from the Americas. Nevertheless, doubts remain with respect to the records of S. grandis and S. spectabilis: the

<table>
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<tr>
<th>Character</th>
<th>N</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>Egg bare diameter</td>
<td>2</td>
<td>126.0–134.0</td>
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<td>Process height</td>
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Table 3. Measurements (in μm) of selected morphological structures of the eggs of Sisubiotus hakaiensis sp. nov. mounted in Hoyer’s medium. Abbreviations: N = number of eggs/structures measured; Range = the smallest and the largest structure among all measured specimens; SD = standard deviation.
considerable distance to their respective type localities and geographical distribution could be indicative of separate (and likely cryptic/pseudocryptic) species being present in the Americas.

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VECCHI M. et al., A new Sisubiotus species from Canada


Mutterer J. & Zinck E. 2013. Quick-and-clean article figures with FigureJ. *Journal of Microscopy* 252: 89–91. [https://doi.org/10.1111/jmi.12069](https://doi.org/10.1111/jmi.12069)


Stec D., Smolak R., Kaczmarek Ł. & Michalczyk Ł. 2015. An integrative description of *Macrobiotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiontidae: *hufelandi* group) from Kenya. *Zootaxa* 4052: 501–526. [https://doi.org/10.11646/zootaxa.4052.5.1](https://doi.org/10.11646/zootaxa.4052.5.1)


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Supplementary files

Supp. file 1. SM.01. Morphometric data for *Sisubiotus hakaiensis* sp. nov.
https://doi.org/10.5852/ejt.2022.823.1815.6991

Supp. file 2. SM.02. Model selection results on the concatenated alignment.
https://doi.org/10.5852/ejt.2022.823.1815.6993

Supp. file 3. SM.03. MrBayes input file for phylogenetic analysis including the used alignment.
https://doi.org/10.5852/ejt.2022.823.1815.6995

Supp. file 4. SM.04. Output phylogenetic tree from MrBayes analysis.
https://doi.org/10.5852/ejt.2022.823.1815.6997

Appendix 1. PCR primers for amplification of the four DNA fragments sequenced in the study.

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### Appendix 2. GenBank accession numbers of DNA sequences used in the phylogenetic analysis.

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