A NEW SPECIES OF *BRYOCYCLOPS* (COPEPODA: CYCLOPIDAE) FROM LEAF LITTER IN THE LAUREL FORESTS OF MADEIRA ISLAND, PORTUGAL

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Abstract.— Wet litter can harbour a diverse copepod fauna, and plays a not yet fully understood role in the dispersal of groundwater organisms. A new species of *Bryocyclops*, a circumtropical genus occurring in cave and semiterrestrial habitats, has been found in the leaf litter of Laurisilva, the native and relict forests on Madeira Island. *Bryocyclops laurisilvae* **sp. nov.** belongs to a species assemblage traditionally named as Group I in the genus. The new species can be distinguished from other species by several apomorphic features, such as the sexually dimorphic setation of the endopodites of leg 3 and leg 4, and the oligomerized maxilliped. A sister relationship between *B. laurisilvae* **sp. nov.** and stygobiotic *B. absalomi* Por, 1981 (Israel) is supported by, among others, the apomorphies of leg 3 in male and leg 4 in female. The new species differs from *B. absalomi* in the presence of coxopodite seta on leg 1, the shorter dorsal and longer posterolateral and outer terminal caudal setae, and the acute, hook-like tip of the apical spine on the distal endopodite segment of leg 3 in the male. The relationships of the *laurisilvae-absalomi* clade remains ambiguous, as the limb morphology in many species is still insufficiently understood.

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INTRODUCTION

Bryocyclops are minute (0.3–0.7 mm) tropical freshwater crustaceans living in cryptic environments, such as wet mosses, phytotelmata, leaf litter, tree hole and dripping pool in cave, as well as in ephemeral anthropogenic habitats (e.g. ditches, tin cans) (Reid 2001, Watiroyram *et al.* 2012). Due to the small body size and apparent rarity of some species known only from old descriptions, basic information on limb morphology in many species is still lacking. As a result, the taxonomic relationships within the genus and between *Bryocyclops* and other genera are still poorly understood. The diagnostic criteria used to define *Bryocyclops*, and the composition of the genus have been discussed for decades (Lindberg 1954a, 1956, Monchenko 1972, Rocha *et al.* 1998, Reid 1999, Fiers 2002, Fiers and Van Damme 2017, Watiroyram 2021). The subgroups defined and named by Lindberg (1954a, 1956) as 'V' and 'VI' are now considered separate genera, *Rybocyclops* Dussart, 1982 and *Haplocyclops* Kiefer, 1952, respectively. In a discussion of the generic characters, Watiroyram (2021) proposed removing two other groups, Group III (*B. constrictus* Lindberg, 1947; *B. travancoricus* Lindberg, 1947) and Group IV (*B. africanus* Kiefer, 1932) from *Bryocyclops*. Fiers and Van Damme (2017) erected the genus *Thalamocyclops* to accomodate *T. pachypes* Fiers & Van Damme, 2017 and *T. soqotraensis* (Mirabdullayev, Van Damme & Dumont, 2002) originally assigned to the genus *Bryocyclops*. On the other hand, a new group has been defined by Watiroyram *et al.* (2015), which currently includes two stygobiotic species from Thailand, *B. maholarnensis* Watiroyram, Brancelj & Sanoamuang, 2015 and *B. jayabhumi* Watiroyram, 2021. Recent studies discovering six new species in cave pools in Thailand alone (Watiroyram *et al.* 2012, 2015, Watiroyram 2018 a,b, 2021), indicate that the actual diversity in this genus can be much higher than is currently known.

Bryocyclops (~20 spp.) with only two Neotropical representatives on the Atlantic coast of Brazil and Puerto Rico occur mainly in the Old World (East Africa, Madagascar, Middle East and Southeast Asia) and Pacific islands (Vanuatu, Guam, Hawaii, Fiji, Tonga and Western Samoa) (Dussart and Defaye 2006, Watiroyram 2021). North American (Florida) populations of the Asian *B. muscicola* (Menzel, 1926) could likely be transported by humans with tropical plants (Reid 1999, Bruno *et al.* 2005, Reid and Hribar 2006, 2019). The large species ranges observed in *B. anninae* (Menzel, 1926), *B. bogoriensis* (Menzel, 1926), *B. caroli* Bjornberg, 1985 and *B. fidjiensis* Lindberg, 1954, however, indicate that at least those *Bryocyclops* may be good dispersers (Yeatman 1983, Reid 1999, Dussart and Defaye 2006). Insular species

(present, but not necessarily endemic to an island) account for about 40% (8 of 20) of the global *Bryocyclops* fauna, while the average proportion of island species is 27% in the speciose genera of Cyclopidae (Hołyńska and Sługocki 2023). Also, *Bryocyclops* occur both on the near-continent (Sumatra, Java and Bali) and remote oceanic islands (Kiefer 1933, Lindberg 1954b, Yeatman 1983, Dussart and Defaye 2006).

Semiterrestrial habitats, such as wet leaf litters, are home to a great variety of harpacticoid copepods and also some cyclopid (including four *Bryocyclops*) species in both tropical and temperate regions (Reid 1999, 2001, Fiers and Ghenne 2000, Watiroyram *et al.* 2012, Brancelj and Karanovic 2015). All *Bryocyclops* species (*B. anninae*, *B. phyllopus* Kiefer, 1935, *B. caroli* and *B. muscicola*) found in leaf litter have been reported from tropical/subtropical regions (Bjornberg 1985, Defaye and Heymer 1996, Reid 1999, 2001). The discovery of stygobiotic copepods in the wet soil of deciduous forests and grasslands in Belgium, and the largely congruent geographic distributions of the European beech (*Fagus sylvatica* Linnaeus, 1753) and subterranean cyclopid, *Graeteriella unisetigera* (Graeter, 1908), prompted Fiers and Ghenne (2000) to propose a hypothesis that moist leaf carpet acts as "means of dispersion to uncolonized aquifers", and as such helps maintain the genetic continuity between isolated subterranean habitats. It is reasonable to expect that (sub)tropical forest litter may harbour a similar or even greater diversity of copepods, and that its role in the species range expansion is not less important than in the temperate zone.

Laurel forests (Laurisilva) are evergreen, subtropical forests with mild temperature, high rainfall and patchy geographic distribution but are found almost all over the world. The Macaronesian laurel forests have been traditionally considered as a 'Tertiary relict' biome widely distributed in Europe until the climate cooling in the Late Miocene (but see Kondraskov *et al.* (2015) advocating a younger Pliocene–Pleistocene origin in majority of the plant species on the Macaronesian islands). Laurisilva occurs in mountain cloud belts (500–1500 m a.s.l.) in the Azores, Canary Islands and Madeira, of which the latter has more than half of the total area of the Macaronesian laurel forests (Guimarães and Olmeda 2008).

Madeira is a volcanic island mostly formed by Pliocene to Pleistocene alkalic basalt lava flows, with the oldest subaerial rocks dating back to 4.6 million years (Geldmacher *et al.* 2000). Ninety percent of the island is above 500 m; max. altitude reaches 1,860 m a.s.l. (Baioni 2011). The climate is humid subtropical: the average annual temperature typically ranges from 17°C to 19°C, with notable variations with altitude (Baioni 2011); the annual precipitation varies from 600–800 mm on the south coast to 3000 mm in the

central high areas (Bica da Cana) (Prada *et al.* 2009, Baioni 2011). Orographic fog persists for more than 200 days per year at elevations between 800 m and 1600 m (Prada *et al.* 2009).

Extensive collections of the freshwater microcrustaceans in lentic and lotic habitats in (semi)natural and anthropogenic areas on Madeira revealed a higher diversity (14 including two non-native species) of the cyclopid copepods than predicted by the island biogeography models (Hołyńska and Sługocki 2023, Sługocki *et al.* in press). Sługocki *et al.* (in press) found also that some rare cyclopid and harpacticoid species were found in the native forest habitats and could be associated with groundwaters. During the expedition we also conducted a pilot study on the litter fauna of the Laurisilva in the Madeira Natural Park/World Heritage Site by UNESCO. Based on that material we report here on the discovery of a new species from a tropical genus, *Bryocyclops*, in a mid-latitude island, Madeira.

MATERIALS AND METHODS

The sampling site (Fig. 1) is located approximately 930 meters above sea level. The mean annual rainfall (fog water is not included) was between 1550 and 1800 mm at our sampling site in 1937–2016 (Espinosa *et al.* 2019). Samples (22) were collected in 22 sampling quadrats, covering an area of ca 6400 m², from the top layer of the wet leaflitter with 10 cm thickness (0.5 dm³ each sample) in Laurisilva forest [*Clethra arborea* Aiton, 1789, *Erica platycodon* subsp. *maderincola* (D.C. McClint) Rivas Martínez, Capelo, Costa, Lousã, Fontinha, Jardim & Sequeira, 2002, *Ilex perado* subsp. *perado* Aiton 1789, *Laurus novocanariensis* Rivas Martínez, Lousã, Fernández Prieto, Días, Costa & Aguiar, 2002, *Ocotea foetens* (Aiton) Baillon 1870, *Picconia excelsa* Candolle, 1844, *Persea indica* Sprengel, 1825, *Vaccinium padifolium* Hochstetter ex. Steudel 1841]. Sample numbers refer to individual quadrats at the sampling site (see Fig. 1).

Copepods were extracted from leaflitter using the decantation and sieving method followed by the centrifugal flotation method (van Benzooijen 2006). They were heat killed using tap water, fixed in DESS solution (dimethyl sulphoxide, disodium EDTA and saturated NaCl, used to preserve soil samples; see Yoder *et al.* 2006), and subsequently transferred to 95% ethyl alcohol. The majority (18 of 22) of the samples contained copepods: either only harpacticoids (13 samples), or harpacticoids together with cyclopids
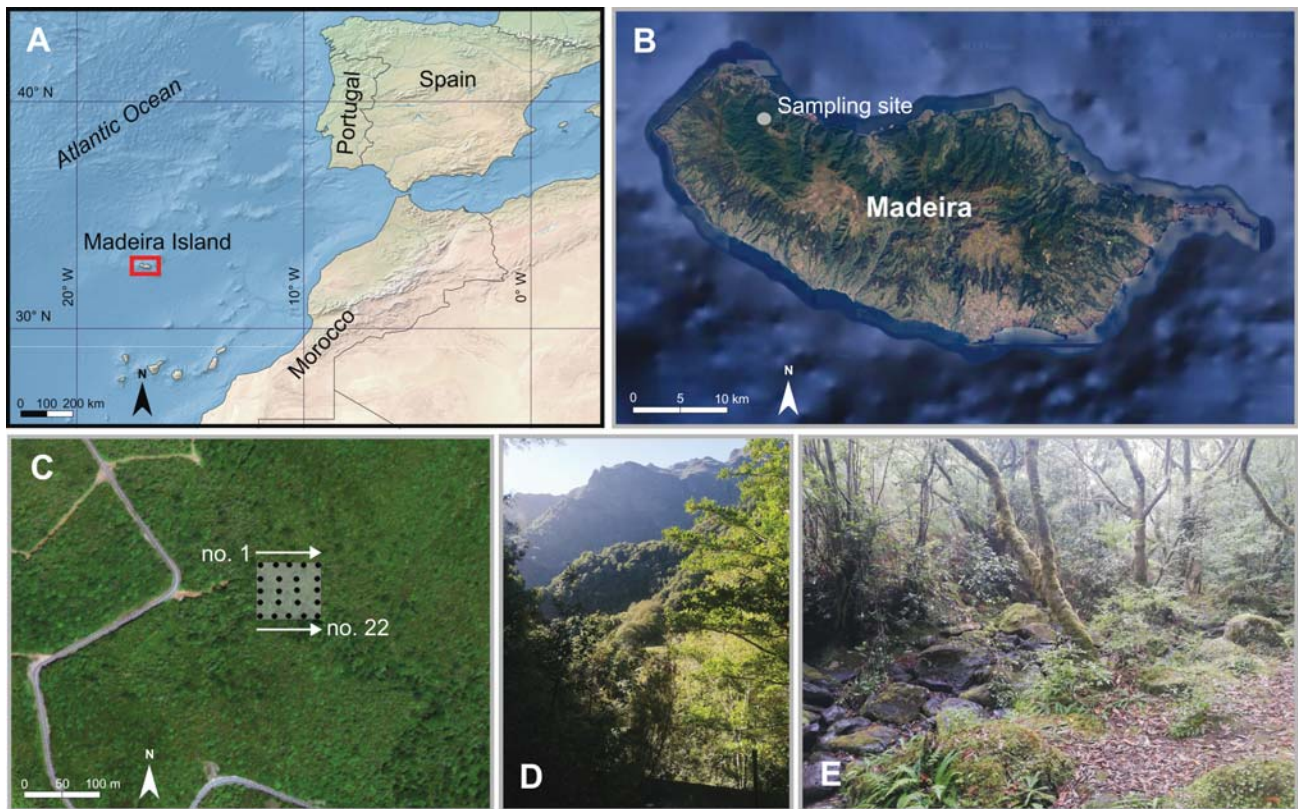

Figure 1. Geographical location of the type locality (A–B), distribution of the sampling quadrats (C), and the laurel forest habitat (D–E) in which *Bryocyclops laurisilvae* sp. nov. was found in Madeira (Portugal).

(5 samples). Selected specimens were fully dissected in glycerine and preserved on microscope slides sealed by nail polish. Telescoping somites were measured separately and summed for total body length (Koźmiński 1936). Pencil drawings were made using a camera lucida attached to Olympus BX 50 compound microscope. Digital drawings were created based on the scanned pencil drawings by CorelDRAW 2018 software.

In the comparisons to the putative close relative of *B. laurisilvae* sp. nov, *Bryocyclops absalomi* Por, 1981, only the original description could be used, as the type material deposited at Hebrew University of Jerusalem, Israel was not available to us.

Morphological abbreviations (numbering of the caudal setae follows Huys and Boxshall 1991):
ae – aesthetasc,
Caudal seta II – anterolateral,
Caudal seta III – posterolateral,
Caudal seta IV – outer terminal,
Caudal seta V – inner terminal,
Caudal seta VI – terminal accessory,
Caudal seta VII – dorsal,
enp 1–2 – first to second endopodite segment,
exp 1–2 – first to second exopodite segment,
P1–P6 – first to sixth legs,
s – seta
sp – spine.

Repositories:
  MIZ – Museum and Institute of Zoology Polish Academy of Sciences, Warsaw, Poland;
  MMF – Natural History Museum in Funchal, Funchal, Portugal.

Results

Phylum **Arthropoda** von Siebold, 1848
Subphylum **Crustacea** Brünnich, 1772
Subclass **Copepoda** Milne-Edwards, 1840
Order **Cyclopoida** Burmeister, 1835
Family **Cyclopidae** Rafinesque, 1815

Genus *Bryocyclops* Kiefer, 1927

Type species. *Bryocyclops anninae* (Menzel, 1926).

Bryocyclops laurisilvae sp. nov.

Etymology. The specific name refers to the type of habitat, laurel forest (Laurisilva in Latin), where the new species has been found. 'Laurisilvae' is a feminine singular noun in genitive case, meaning "of the laurel forest".

Type material. Holotype: ♀ (fully dissected), Portugal, Madeira Island, Madeira Natural Park/World Heritage Site by UNESCO, Laurisilva forest, 32°49'15.5"N 17°09'09.1"W, sample 7, 25 Sep. 2019, Ł. Sługocki leg., washed from leaflitter, dry soil, top layer of leaflitter was wet after rain, MIZ PAN CRU 001207. Paratypes: 1♀ and 2♂♂ (fully dissected), collection data as in the holotype, MIZ PAN CRU 001208, MIZ PAN CRU 001209 and MMF: 50279; 1♂ (fully dissected), sample 6, other data as in holotype, MIZ PAN CRU 001210; 1♂ (undissected, preserved in 95% ethyl-alcohol), sample 12, other data as in holotype, MIZ PAN CRU 001211; 2♂♂ (undissected, preserved in 95% ethyl-alcohol), sample 13, other data as in holotype, MIZ PAN CRU 001212, MIZ PAN CRU 001213; 1♂ (undissected, preserved in 95% ethyl-alcohol), sample 18, other data as in holotype, MMF: 50280.

Diagnosis. The following combination of characters separate the new species from all its congeners (unless otherwise stated, character states apply to both sexes). Antennule 11-segmented in female. Maxilliped three-segmented, with one, one and three setae, respectively. P1–P4 with two-segmented exp and enp. Spine and seta formula of terminal exopodite segments of swimming legs, 3,3,3,3 and 5,5,5,4, respectively. Intercoxal sclerites bearing large triangular protuberances in P1–P4. Coxopodite seta present in P1 and absent in P2–P4. Medial setae of P4 enp2 robust and only slightly (1.4–1.7 times) longer than apical spine in female. P5 composed of three setae inserted on pediger 5. Anal operculum reaching beyond level of insertion of seta II, triangular, free margin denticulate. Caudal seta II inserted near half-length of caudal ramus. In male, P3 enp2 armed with four medial setae, of which two distal ones highly reduced in length (shorter than half-length of more proximal setae); segment apically bearing semicircular protuberance between insertions of lateral seta and modified apical spine; modified apical spine with distinct denticulate outgrowth at half length, tip of spine hook-like and acute.

Description. **Female.** Body length (excluding caudal setae) 465–490 μm (n = 2) (Fig. 2A). Prosome length/urosome length 2.0–2.2; cephalothorax nearly as long as wide; width of cephalothorax/width of genital double-somite 1.6–1.7. Refractile points, observed as dark dots by light microscopy, present on all prosomal somites, pediger 5 and genital double-somite. Genital double-somite (Fig. 2B–C) 1.6–1.8 times wider than long. Two kidney-shaped spermatophores with distinctly thick wall, connecting to each other along long axis, and attached to ventral surface in both females. Pair of egg sacs with two large (diameter, 59 μm) eggs each. Genital double-somite and succeeding two urosomal somites with denticulate hyaline frills, incisions
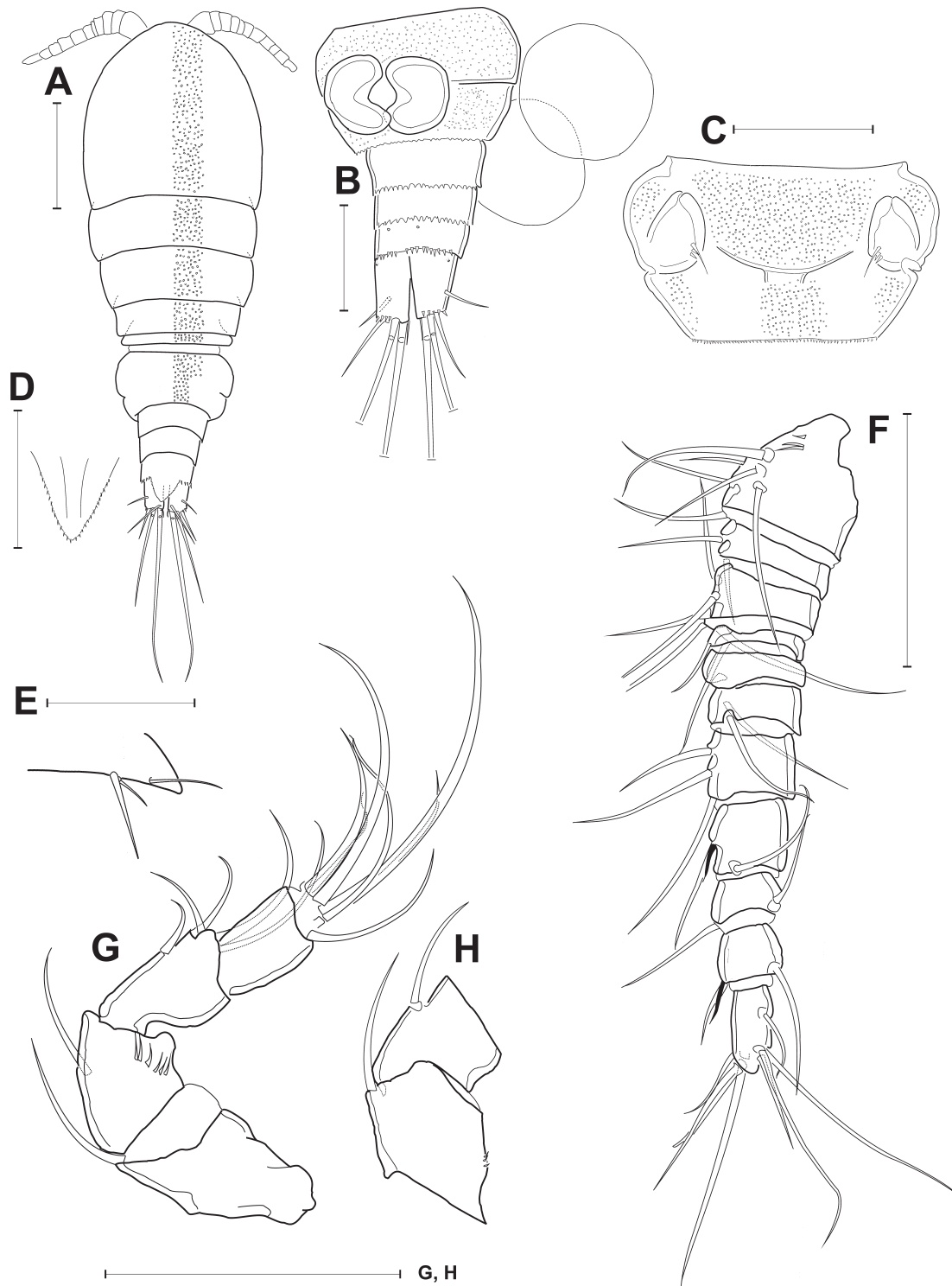
Figure 2. *Bryocyclops laurisilvae* sp. nov., female: (A) habitus, dorsal view, refractile points are partially illustrated; (B) urosome with two spermatophores, egg sac with two eggs illustrated on only one side, ventral view; (C) genital double-somite, dorsal view; (D) anal operculum dorsal view, note dorsal keel in middle; (E) pediger 5 in part, and P5, ventral view, refractile points are not shown; (F) antennule, ventral view; (G) antenna, frontal view; (H) coxobasipodite and first endopodite segment of antenna, caudal view. B and E show the holotype, other figures show paratype MIZ PAN CRU 001208. Scale bars: A = 100 μm; B–H = 50 μm.

deeper on more posterior somites. Anal somite (Fig. 2B) with robust spinules on posterior margin; two medial pores in anterior half and two medial sensilla present on ventral and dorsal surfaces, respectively. Anal operculum (Fig. 2A,D) large, almost reaching or extending slightly beyond posterior margin of caudal rami (verified in dorsal view); triangular with obtuse tip, tiny dents present on whole margin except for most proximal part; distinct, wide keel present in middle section. Caudal ramus (Fig. 2B) 1.7–1.8 times as long as wide, medial margin without hairs. Dorsal keel absent or not distinct. Robust spinules present at insertion of caudal seta III, spinules absent at insertion of seta II. Seta II 0.66–0.75 times as long as caudal ramus (n = 2), inserted at posterior 4/10 of ramus length. Seta VI reaching fracture plane of seta V, ca 1/4 to 1/3 of length of seta III and 0.3–0.4 times as long as caudal ramus (n = 2). Caudal setae VII, V and IV 1.4, 5.3 and 3.1 times as long as seta III, respectively (length proportions were verified in the paratype as the setae were partly injured in the holotype). Seta III ca 1.1 times as long as caudal ramus length (n = 2). Seta VII inserted at posteromedial angle of ramus.

Antennule (Fig. 2F) 11-segmented, not reaching posterior margin of cephalothorax. Setation formula: 1(I–V): 6 s; 2(VI–VII): 2 s; 3(VIII–XI): 5 s; 4(XII–XIII): 2 s; 5(XIV): 1 sp; 6(XV–XVI): 2 s; 7(XVII–XX): 3 s; 8(XXI–XXIII): 2 s +1 ae; 9(XXIV): 2 s; 10(XXV): 2 s+ 1 ae; 11(XXVI–XXVIII): 7 s +1 ae. Apical acrothek, consisting of one seta and one aesthetasc, present on last antennulary segment. First segment bearing few long spinules on anterior (ventral) surface.

Antenna (Fig. 2G–H). Four-segmented, comprising coxobasipodite and three-segmented endopodite, and bearing 1, 1, 5 and 7 setae, respectively. Exopodite and inner medial (inserted on caudal surface) setae are absent on first segment (coxobasipodite). Coxobasipodite without ornamentation, except for few tiny spinules on lateral margin in proximal half of segment.

Labrum (Fig. 3A) with 12 teeth on posterior margin, and smooth triangular lobes laterally to teeth. Fringe hairs present on ventral surface and arranged in 1–1 arc. Mandible (Fig. 3B) with single short seta on palp, no surface ornamentation on frontal surface of mandibulary coxopodite. Maxillule (Fig. 3C) armed with three apical claws, one seta on caudal surface, six articulated setae on medial margin of precoxal arthrite, and one short unarticulated spine near proximal margin (indicated by arrowhead). Maxillulary palp (Fig. 3D) naked, with one proximal and three apical setae, lateral lobe bearing three setae. Maxilla (Fig. 3E) composed of syncoxopodite (division between praecoxopodite and coxopodite present only on caudal surface), basipodite and two-segmented endopodite. Praecoxal endite bearing two setae, proximal and distal endites of coxopodite with one and two setae, respectively. Basipodite with two setae: long spinous seta inserted in front of claw-like medial extension of basipodite, with tiny setules on convex margin in distal 2/3 of seta; and short seta inserted on caudal surface. Two-segmented endopodite bearing two and three setae, respectively. Maxilliped (Fig. 3F) three-segmented, with one, one and three setae, respectively. Syncoxopodite with distinctly long spinules on lateral margin. Basipodite bearing longitudinal row of slender spinules on frontal surface and transverse row of spinules at laterodistal angle on caudal surface. Setae on endopodite segment subequal in length.

P1–P4 (Fig. 4A–G and Table 1) with two-segmented rami. Precoxopodite distinct in all swimming legs, wide triangular in P1–P3 and oval shape in P4. Coxopodite seta present in P1 (Fig. 4A) and absent in P2–P4 (Fig. 4B,D,F). Small acute outgrowth present at laterodistal angle of coxopodite on caudal surface in P1–P4. Oblique row of long spinules (Fig. 4B,D) proximally to acute outgrowths present in P2 and P3 on caudal surface; P1 and P4 coxopodites without surface ornamentation. Intercoxal sclerites naked and bearing large triangular protuberances in P1–P4, tip of protuberances caudally slightly curved in P4 (Fig. 4F). P1 basipodite (Fig. 4A) with robust medial spine reaching distal margin of P1 enp1. Medial expansion of basipodite apically rounded in all swimming legs, and medially pilose in P1–P3 yet naked in P4. Blunt and robust setae with slightly curved tip present on exp2 of P1–P3: third seta in P1 (Fig. 4A, indicated by arrowhead), and second and third setae in P2 and P3 each (Fig. 4C–D, indicated by arrowheads). Setae on P4 enp2 (Fig. 4G) robust and only slightly longer than apical spine (relative length is given in relation to length of apical spine

Table 1. Armature of legs 1–4 in *Bryocyclops laurisilvae* sp. nov. Spines are denoted by Roman numerals, setae by Arabic numerals. The armature on the lateral margin of any segment is given first, followed by the elements on the apical and medial margins.

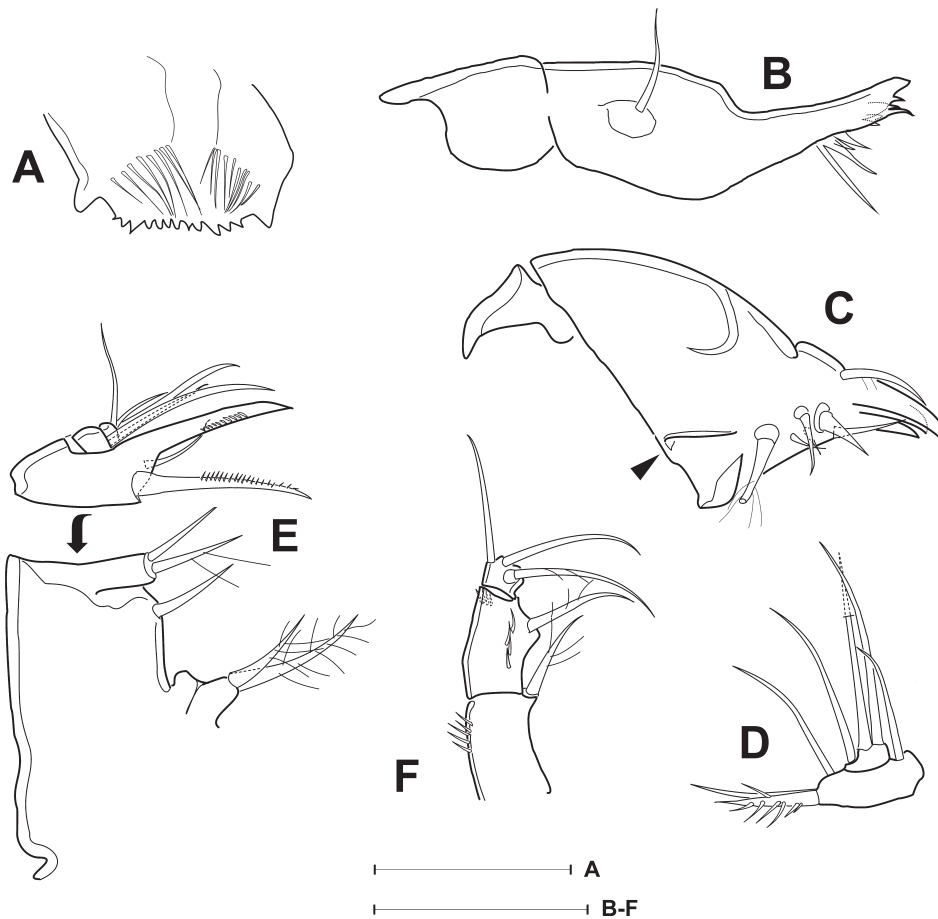|	Coxopodite	Basipodite	Exopodite	Endopodite
Leg 1	0-1	1-I	I-0; III-2-3	0-1; 1-I,1-1
Leg 2	0-0	1-0	I-0; III-2-3	0-1; 1-I,1-1
Leg 3	0-0	1-0	I-0; III-2-3	0-1; 1-I,1-3
Leg 4	0-0	1-0	I-0; III-2-2	0-1; 1-I,1-1

Figure 3. *Bryocyclops laurisilvae* sp. nov., female: (A) labrum; (B) mandible, frontal view; (C) maxillule, caudal view, arrowhead points to proximal spine; (D) maxillulary palp (missing part of the median seta on the lateral lobe is illustrated based on the corresponding seta of the maxillule on the opposite side); (E) maxilla, frontal view; (F) maxilliped, frontal view. A and C–F show holotype, B shows paratype MIZ PAN CRU 001208. Scale bars: A–F = 50 μm.

from lateralmost to medialmost setae): 1.2–1.3, 1.0 (apical spine), 1.4–1.7 and 1.5–1.7 (n = 2). P4 enp2 1.0–1.1 times as long as wide, apical spine ca 0.8 times as long as P4 enp2 (n = 2). P4 enp1 1.7–1.8 times wider than long (n = 2). P5 (Fig. 2E) represented by three setae inserted on pediger 5; relative length of setae from medialmost to lateralmost, 1.15, 0.50, 1.0, respectively (measured in holotype). Medialmost and middle short seta inserted next to each other. P6 (Fig. 2C) composed of three elements inserted laterodorsally at midlength of genital double-somite: one seta (12 μm) and two small subequal spines (2.5 μm and 2.0 μm) (measured in paratype).

Male. Body length 440–465 μm (n = 5) (Fig. 5A–D). Prosome relatively shorter than in female: length of prosome/length of urosome 1.5–1.6. Refractile points present on all prosomal somites, pediger 5 (on dorsal surface) and two succeeding urosomites. Shallow cuticular pits (Fig. 5A, indicated by arrowheads) present on dorsal surface of urosomite 4 (few large laterally)

and urosomite 5 (small pits in transverse row). Anal operculum (Fig. 5A,C,D) reaching posterior margin of caudal rami or shorter yet extending beyond insertion of seta II, triangle/semi-ellipse shape, tip acute or rounded, tiny dents present on margin, wide keel present in middle section. Caudal ramus (Fig. 5C–D) 1.7–1.9 times as long as wide (n = 7), dorsal keel absent. Spinules present at insertion of seta III, and absent at insertion of seta II. Seta II 0.75–0.94 times as long as caudal ramus (n = 5), and inserted at distance of 0.38–0.46 ramus length measured from posterior end (n = 6). Seta VII 0.95–1.1 times as long as seta III (n = 3). Seta VI 0.32–0.39 times as long as seta III (n = 6), and 0.40–0.48 times as long as caudal ramus (n = 7). Seta V 5.1 times longer than seta III (single male, MIZ PAN CRU 001211, measured). Seta IV injured in all males. Seta III 1.2–1.3 times as long as caudal ramus (n = 6).

Antennule (Fig. 5E–G). 17-segmented, setation formula: 1(I–V): 7 s + 3 ae; 2(VI–VII): 4 s; 3(VIII): 2 s; 4(IX): 2 s +1 ae; 5(X): 1 s; 6(XI): 2 s; 7(XII): 2 s; 8(XIII):
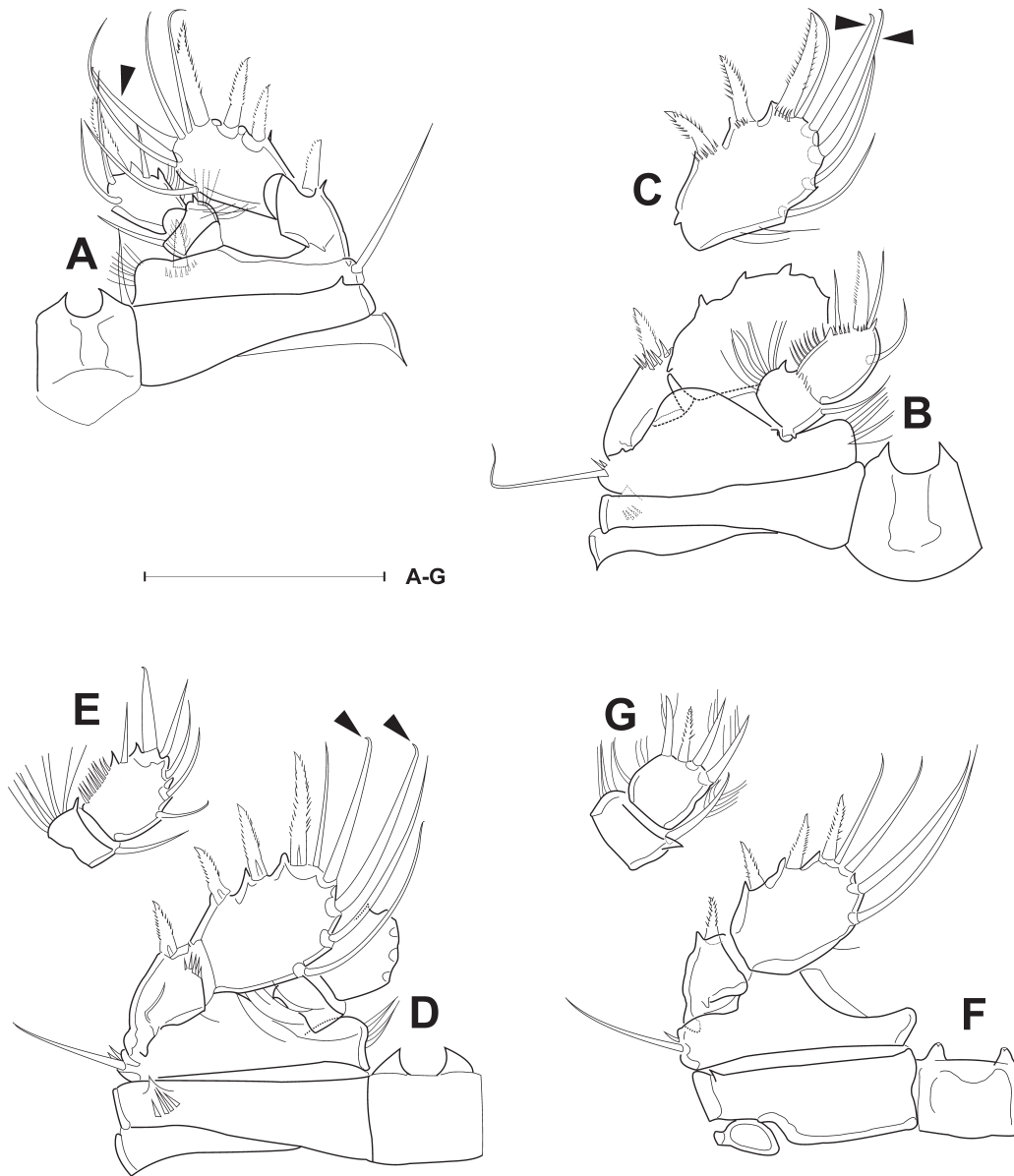
Figure 4. *Bryocyclops laurisilvae* sp. nov., female: (A) leg 1, caudal view; (B) leg 2, frontal view; (C) distal exopodite segment of leg 2, frontal view; (D) leg 3, caudal view; (E) leg 3 endopodite, caudal view; (F) leg 4 (without endopodite), caudal view; (G) leg 4 endopodite, caudal view. Arrowheads point to modified setae. Plumose setulation of the setae is shown only on P4 endopodite. All drawings show the holotype. Scale bar: A–G = 50 μm.

2 s; 9(XIV); 1 sp + 1 ae; 10(XV) 2 s; 11(XVI) 2 s; 12(XVII) 2 s; 13(XVIII) 2 s + 1 ae; 14(XIX–XX): 1 s + 1 plate-like modified seta + 1 small cone-like element; 15(XXI–XXIII): 1 s + 2 plate-like modified setae +1 ae; 16 (XXIV–XXV): 4 s; 17(XXVI–XXVIII): 7 s + 1 ae. Presence of aesthetasc on segment 16, inserted next to short laterodistal seta, could not be unequivocally confirmed. Medial seta of 17th antennulary segment inserted next to proximal margin of segment. First antennulary segment bearing long spinules on anterior (ventral) surface. Segmentation and setation of antenna,

mandible, maxillule, maxilla and maxilliped as in female.

Armature formula of P1–P4 as in female (Table 1), stout and blunt setae present on terminal exopodite segments in P1–P3 in same positions as in female. Intercoxal sclerites naked and bearing large acute protuberances in P1–P4. Medial expansion of basipodite pilose in all swimming legs. P3 enp2 with sexual dimorphism (Fig. 6B cf. Fig. 4E): segment bearing semi-circular protuberance between insertions of modified apical spine and lateral seta; apical spine with distinct
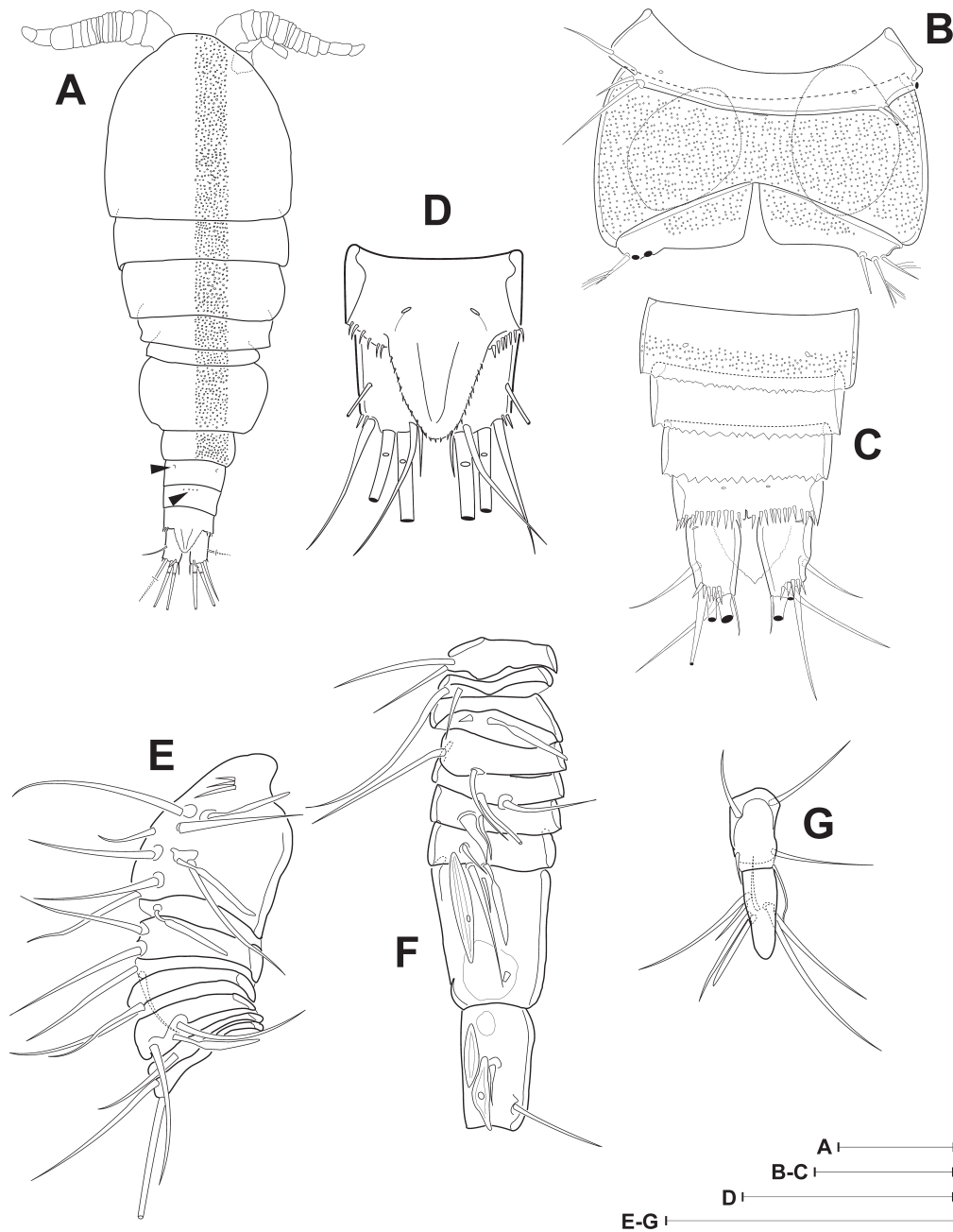
Figure 5. *Bryocyclops laurisilvae* sp. nov., male: (A) habitus, dorsal view, refractile points are partially illustrated, arrowheads point to cuticular pits; (B) pediger 5 and genital segment, ventral view; (C) third to sixth urosomites and caudal rami, ventral view; (D) anal somite and caudal rami, dorsal view, note rounded tip and dorsal keel of anal operculum. E–G. Antennule, ventral view: (E) segments 1–6; (F) segments 7–15; (G) segments 16–17. A shows paratype MMF: 50279, B and C show paratype MIZ PAN CRU 001209, D shows paratype MMF: 50280, E–G show paratype MIZ PAN CRU 001210. Scale bars: A = 100 μm; B–G = 50 μm.

denticulate outgrowth at half length, tip of spine hooklike and acute; two medial setae inserted next to modified spine highly reduced in length (semicircular protuberance of segment and reduced setae are indicated by arrowheads in Fig. 6B). Two long proximal setae on medial margin of P3 enp2 plumose, seta on lateral margin naked. Relative lengths of P4 enp2 setae (Fig. 6C–D) in relation to length of apical spine different from those in female: 1.3–1.7, 1.0 (apical spine), 2.1–2.5, and 2.1–3.0 (n = 4). P4 enp2 ca 1.4 times as long as wide, apical spine 0.7–0.9 times as long as P4 enp2 (n = 4); lateral margin of segment bearing short and
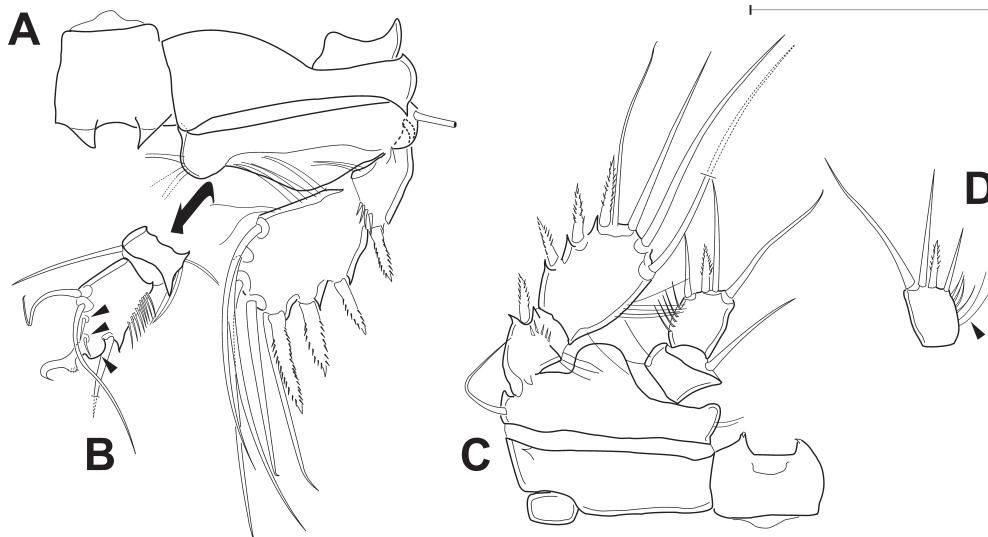
Figure 6. *Bryocyclops laurisilvae* sp. nov., male: (A) leg 3 (without endopodite), caudal view; (B) leg 3 endopodite, arrowheads point to putative apomorphic features, caudal view (missing part of the lateral seta of P3 enp2 is illustrated based on the corresponding seta of P3 on the opposite side); (C) leg 4, caudal view (missing part of the proximalmost seta on P4 exp2 is illustrated based on the corresponding seta in paratype MMF: 50279); (D) second endopodite segment of leg 4 on the opposite side, arrowhead points to long hairs which can be present or absent on the lateral margin. All drawings show paratype MIZ PAN CRU 001209. Scale bar: A–D = 50 μm.

stiff setules (Fig. 6C), or short and stiff and long and fine setules (Fig. 6D). P4 enp1 1.3–1.7 times wider than long (measured across widest part; n = 3). P5 (Fig. 5B) consisting of three setae inserted on pediger 5; medial seta reaching ca half length of genital segment, and 2.1–2.7 and 1.8–2.1 times as long as median and lateral setae, respectively (n = 4). P6 flap with three elements (Fig. 5B): medial seta 25–31 μm long (distal part of medial seta shown in Fig. 5B is probably broken off), and 1.7–2.2 (n = 4) and 2.1–2.3 (n = 2) times as long as median and lateral setae, respectively.

Taxonomic comparisons. *Bryocyclops laurisilvae* sp. nov. belongs to 'Group I' in *Bryocyclops sensu stricto* (Watiroyram 2021), defined among others by the two-segmented states of both rami of P4 in both sexes, and the presence of four elements (1 spine + 3 setae) on P4 enp2 (Lindberg 1954a, 1956, Watiroyram *et al*. 2015, Watiroyram 2021). The group currently includes 11 species: *B. absalomi* (Israel), *B. ankaratranus* Kiefer, 1955 (Madagascar), *B. anninae* (Vietnam, Java, Guam, Hawaii and Vanuatu), *B. apertus* Kiefer, 1935 (Kenya), *B. asetus* Watiroyram, 2018 (Thailand), *B. chappuisi* Kiefer, 1928 (Java), *B. difficilis* Kiefer, 1935 (Kenya), *B. elachistus* Kiefer, 1935 (Kenya), *B. maewaensis* Watiroyram, Brancelj & Sanoamuang, 2012 (Thailand), *B. mandrakanus* Kiefer, 1955 (Madagascar) and *B. phyllopus* Kiefer, 1935 (Kenya, Ethiopia, Democratic Republic of Congo) (Lindberg 1954a,b, 1956, Dussart and Defaye 2006, Watiroyram *et al*. 2012, Watiroyram 2018b, Sanoamuang *et al*. 2019).

By the peculiar morphology of P3 enp2 in male, shared only by *B. absalomi*, the new species can be distinguished from many representatives in group I (comparisons could be made only for those species for which the published illustrations provided detailed information on P3 enp2 in male). Differences are in the number and the size reduction of the medial setae, the shape of the apical spine, and the segment itself. P3 enp2 has four setae on the medial margin, and the length of the two more distal setae are similar to those of the more proximal ones or just slightly reduced (longer than half of the length of the more proximal setae) in *B. ankaratranus*, *B. anninae*, *B. asetus*, *B. mandrakanus*, and *B. phyllopus* (Kiefer 1937, 1939, 1955, 1956, Defaye and Heymer 1996, Watiroyram 2018b, Sanoamuang *et al*. 2019). P3 enp2 is bearing three medial setae, of which the distalmost one is slightly shorter than other setae of the segment in *B. maewaensis* (Watiroyram *et al*. 2012). Also, the rounded apical protuberance of P3 enp2 extending between the insertions of the lateral seta and apical spine and present in male in *B. laurisilvae* and *B. absalomi*, is absent in all the species mentioned above.

The new species differs from *B. elachistus* in the relative length of P4 enp2 appendages in the female: the medial setae and apical spine are distinctly short in *B. laurisilvae* (vs medial setae are ca twice as long as apical spine, and apical spine is longer than segment in *B. elachistus*) (Kiefer 1937, 1939). Concerning the male morphology, *B. laurisilvae* can be distinguished

from *B. elachistus* and *B. difficilis* by the longer caudal rami (vs length/width ca 1.5 or less in *B. elachistus* and *B. difficilis*), triangle/semi-ellipse shape of anal operculum (vs semicircular in *B. elachistus* and *B. difficilis*), and the highly reduced caudal seta VI (vs ca. half as long as seta III in *B. elachistus* and *B. difficilis*) (Kiefer 1935, 1939).

Bryocyclops apertus (only the male is known) differs from the new species in the size and shape of the anal operculum (short, reaching to anterior third of caudal rami, rounded shape and smooth free margin in *B. apertus*; vs long reaching to or beyond insertion of seta II, triangle/semi-ellipse shape, free margin with small dents in *B. laurisilvae*) (see Kiefer 1939).

Bryocyclops chappuisi (only the female is known) differs from *B. laurisilvae* in the morphology of P4 enp2 (segment is ca 1.5 times longer than wide, and the apical spine is at least as long as the article in *B. chappuisi*), insertion of the anterolateral (II) caudal seta (posterior third in *B. chappuisi*) and the short caudal rami (ca 1.5 times as long as wide in *B. chappuisi*) (Kiefer, 1928, 1933).

The most similar *B. absalomi* differs from *B. laurisilvae* sp. nov. in: (1) the absence of coxopodite seta on P1; (2) relative lengths of the caudal setae (seta VII is longer, at least twice as long as seta III; seta IV is shorter, about one-third of the length of seta V and 1.7–1.8 times as long as seta III; and seta III is shorter, ca 0.6 times as long as caudal ramus length); (3) and the shape of the anal operculum (tip acuminate or cuspidate rather than obtuse) (all the character states here mentioned were illustrated by Por 1981 in the female only). Also, illustrations of the male in *B. absalomi* (see Por 1981) reveal the tip of the modified spine on P3 enp2 to be wide blunt rather than hook-like and acute (the latter character state is present in *B. laurisilvae* sp. nov.).

Distribution. Madeira Island.

A preliminary identification key to the species of Group I sensu Lindberg (1954, 1956)

Morphological information about some species is limited to one sex (indicated after species name). Unless otherwise stated, character states apply to both sexes.

1. P4 endopodite one-segmented in female **2**
–. P4 endopodite two-segmented in female . . . . **3 (Group I)**
2. One-segmented P4 endopodite with five elements in female; coxopodite seta present on P1 . . . **Group II**
–. One-segmented P4 endopodite with two elements in female; coxopodite seta absent on P1 . . . . **Group III**
               (=Group VII in Watiroyram *et al.* 2015)

3. Anal operculum with smooth margin **4**
–. Anal operculum with denticulate margin **6**
4. Anal operculum semicircular/semielliptical, posterior end not reaching level of insertion of caudal seta II . . . . ***B. apertus*** Kiefer, 1935 (male only)
–. Anal operculum triangular, posterior end reaching to or beyond level of insertion of caudal seta II . . . **5**
5. Caudal seta VI extending only to fracture plane of seta V, and shorter than half-length of seta III; maxilliped four-segmented, terminal segment with two setae . . . . ***B. anninae*** (Menzel, 1926)
–. Caudal seta VI reaching well beyond fracture plane of seta V, and longer than half-length of seta III; maxilliped three-segmented, terminal segment with three setae . . . . ***B. phyllopus*** Kiefer, 1935
6. Caudal seta II inserted at posterior third of caudal ramus . . . ***B. chappuisi*** Kiefer, 1928 (female only)
–. Caudal seta II inserted more anteriorly, near half-length of caudal ramus **7**
7. Posterior end of anal operculum not reaching level of insertion of caudal seta II . . . . ***B. difficilis*** Kiefer, 1935
–. Posterior end of anal operculum reaching to or beyond level of insertion of caudal seta II **8**
8. Anal operculum semicircular (length and half-width of operculum subequal) in male . . . . ***B. elachistus*** Kiefer, 1935
–. Anal operculum not semicircular (length distinctly greater than half-width) in male (and also in female when they are known) **9**
9. P3 enp2 with three medial setae in male . . . . ***B. maewaensis*** Watiroyram *et al.* 2012
–. P3 enp2 with four medial setae in male **10**
10. Two distal setae on medial margin of P3 enp2 strongly reduced (less than half-length of more proximal setae) in male **11**
–. Two distal setae on medial margin of P3 enp2 not, or slightly reduced in length (longer than half-length of more proximal setae) in male **12**
11. Coxopodite seta absent on P1 (data in female); modified spine on P3 enp2 with wide and blunt tip in male . . . . ***B. absalomi*** Por, 1981
–. Coxopodite seta present on P1 (in both sexes); modified spine on P3 enp2 with hook-like and acute tip in male . . . . ***B. laurisilvae*** sp. nov.
12. Coxopodite seta absent on P1 (in both sexes) . . . . ***B. asetus*** Watiroyram, 2018
–. Coxopodite seta present on P1 (data in male) . . . **13**
13. Protuberance on lateral margin of modified apical spine on P3 enp2 smooth in male . . . . ***B. mandrakanus*** Kiefer, 1955 (male only)
–. Protuberance on lateral margin of modified apical spine on P3 enp2 denticulate in male . . . . ***B. ankaratranus*** Kiefer, 1955 (male only)

Discussion

There is little doubt about the generic and subgroup affiliations of the new species. The Madeiran cyclopid shows all the diagnostic characters of *Bryocyclops sensu* Watiroyram 2021, namely: (1) 11-segmented antennule in female; (2) well developed anal operculum; (3) genital double-somite wider than long; (4) two segmented exo- and endopodites in P1–P4; (5) presence of medial seta/spine on P1 basipodite; (6) sexually dimorphic apical spine on P3 enp2; (7) intercoxal sclerite with large acute protuberances in P4; (8) absence of coxopodite seta in P4 (and in P2–P3, too); (9) spine and seta formula of the terminal exopodite segments in P1–P4 are 3,3,3,3 and 5,5,5,4, respectively; (10) P5 represented by only three setae inserted on pediger 5; (11) P6 with three elements in male.

Bryocyclops laurisilvae sp. nov. has also all the distinguishing features of Group I as defined by Lindberg (1954a, 1956). However, four (characters 5–7 and 9 in the list above) of the seven diagnostic characters of Group I are now those of the genus *sensu stricto* (Watiroyram *et al.* 2015, Watiroyram 2021), while the remaining three (presence of coxopodite seta in P1; two-segmented rami of P4 in both sexes; and presence of one spine and three setae on P4 enp2) are plesiomorphic in *Bryocyclops*. The currently available data do not support monophyly of Group I. We need more information on the limb morphology including the cephalothoracic appendages in both sexes to corroborate the monophyly of Group I. The relationships of *B. ankaratranus*, *B. apertus* and *B. mandrakanus*, so far considered to be members of Group I (Lindberg 1954a, 1956, Dussart 1982), need to be verified as well, as those species are known only from male specimens.

The close (sister) relationship of *B. laurisilvae* sp. nov. and *B. absalomi* is supported by at least two 'losses' and one 'gain' on P3 enp2 in male: the two distal setae on the medial margin are strongly reduced in length; and a rounded apical protuberance is present between the apical spine and lateral seta (Fig. 6B). In a survey of the published illustrations we could not find any other species with similar morphology; therefore the features above are hypothesised to be unique apomorphies of the *laurisilvae–absalomi* clade in *Bryocyclops*. There are also other similarities between these species in the size of anal operculum, presence of a wide keel in the middle section of the anal operculum (Figs 2D, 5A,D), and the reduced length of the medial setae on P4 enp2 in female (Fig. 4G), which, however, are either not unique for the *laurisilvae–absalomi* clade, or the character state is continuous rather than discrete. The intraspecific variation found in the length of the anal operculum in *B. laurisilvae* sp. nov. (see the description and Fig. 5A,C,D), somewhat reduces the diagnostic value of this character. A dorsal keel on

the anal operculum, similar to that in the *laurisilvae–absalomi* clade, to our knowledge has not yet been reported in any other *Bryocyclops*, although this feature may have been overlooked or not illustrated in the old descriptions. Two longitudinal depressions on the dorsal surface of the anal operculum are present in *B. muscicoloides* Watiroyam, 2018 and *B. trangensis* Watiroyram, 2018 (see Watiroyam 2018a), but the shape of the 'crest' between these depressions differs in both species from that in the *laurisilvae–absalomi* clade. Besides, both *B. muscicoloides* and *B. trangensis* belong to another phyletic lineage, Group II (Lindberg 1954a, 1956), having one-segmented P4 endopodite in female. The short medial setae of P4 enp2 (ca as long as segment, and 1.4–1.7 times as long as apical spine) present in the female in *B. laurisilvae* sp. nov. and *B. absalomi*, appear only in *B. maewaensis* (Thailand, cave) in Group I. This character could not be verified in the Eastern African *B. apertus* and Madagascan *B. ankaratranus* and *B. mandrakanus*, as the female is unknown in these species. *Bryocyclops maewaensis* differs from the *laurisilvae–absalomi* clade in: (1) the morphology of the maxilliped (endopodite is two-segmented; syncoxopodite with two setae – no data in *B. absalomi*); (2) P3 enp2 in male (three medial setae rather than four; rounded apical protuberance of the segment is absent); (3) medially pilose P4 basipodite; (4) and the presence of distinctly large dents on the free margin of anal operculum (Watiroyram *et al.* 2012).

Beyond the reduced setae on the swimming legs (P3 enp2, male; P4 enp2, female), the oligomerized maxilliped is a particularly interesting distinguishing characteristic of *B. laurisilvae* sp. nov. In the cyclopine–eucyclopine body plan the maxilliped endopodite is two-segmented from copepodid I to adult instar (Ferrari and Ivanenko 2001), and the 'two-segmented' character state has been found in the adult in several *Bryocyclops* species (*B. anninae*, *B. asetus*, *B. jayabhumi*, *B. maholarnensis*, *B. maewaensis*, *B. muscicoloides*, *B. trangensis*) (Watiroyram *et al.* 2012, 2015, Watiroyram 2018 a,b, Sanoamuang *et al.* 2019). In all *Bryocyclops* with two-segmented maxilliped endopodite, the proximal and distal segments are armed with one and two setae, respectively. The two-setae state on the distal endopodite segment probably is a plesiomorphy in *Bryocyclops sensu stricto*, because this same rather than the three-setae state occurs in the closely related genera, *Haplocyclops* and *Thalamocyclops* (Rocha *et al.* 1998, Fiers 2002, Fiers and Van Damme 2017). In contrast, *B. laurisilvae* sp. nov. has single endopodite segment bearing three setae. Interestingly, this same morphology has been documented in *B. phyllopus* (Eastern Africa) in Group I, and in three species in Group II, *B. campaneri* Rocha et Bjornberg, 1987 (Brazil), *B. caroli*

(Brazil and Puerto Rico) and *B. muscicola* (Java, Sumatra, Thailand, USA – introduced) (Bjornberg 1985, Rocha and Bjornberg 1987, Defaye and Heymer 1996, Reid 1999). From the presence of three setae on the single endopodite segment in the *Bryocyclops* species mentioned above and the setation pattern in the species with two-segmented endopodite, we may infer that the single endopodite segment is a complex segment in which the arthrodial membrane between two endopodite segments failed to form. The presence of the complex endopodite segment is a derived character in *Bryocyclops*, yet its value as phylogenetic signal needs to be tested by the congruent distribution of other morphological features. Also, interpretation of the illustrations themselves can sometimes be problematic in these tiny crustaceans, and verification of the maxilliped morphology may be desirable in *B. campaneri* and *B. caroli* at least.

The presence of a single seta on the maxilliped syncoxopodite seems to be a unique apomorphy of *B. laurisilvae* sp. nov. (or the *laurisilvae–absalomi* clade?), as in all species in which the maxilliped has been described and/or illustrated the syncoxopodite is armed with two setae. Unfortunately in nearly half of the *Bryocyclops* species, including *B. absalomi,* the putative closest relative, there is no information on the maxilliped morphology. In the closely related *Haplocyclops* and *Thalamocyclops* the maxilliped syncoxopodite has one and two setae, respectively (Rocha *et al.* 1998, Fiers 2002, Fiers and Van Damme 2017).

While the habitat data support native status of the Madeiran species, the origin of *B. absalomi* in Soreq Cave in Israel (40 km from the Mediterranean coast, 385 m a.s.l.) is not so obvious. Before its discovery in 1968, the Soreq Cave probably did not have any natural openings, and during the samplings conducted in 1968 no copepods were found in the cave pools (Por 1981). The cave was opened to tourism in 1977, and soon after in 1980 *Bryocyclops* was found in large numbers. Por (1981) surmised that *B. absalomi* is a newcomer in Soreq: "*the changing conditions in the cave enabled this species to invade it (and become dominant there) from some marginal environment where it lived, probably in small numbers.*" That *Bryocyclops* may occur in semi-arid regions in cryptic habitats is not so surprising perhaps, in light of the fact that some close relatives of *Bryocyclops*, *Palaeocyclops* (Kisil-Kum, Central Asia; wells) and *Thalamocyclops* (Socotra Island, Yemen; rock hole, cave) also managed to survive in harsh, dry environments (Monchenko 1972, Fiers and Van Damme 2017). On the other hand, the most recent common ancestor of the *laurisilvae–absalomi* clade may have lived in environments with high humidity and mild temperatures, inferred from the habitats in which *B. laurisilvae* sp. nov. and most extant *Bryocyclops* species were found.

Concerning the geographic origin of *B. laurisilvae* sp. nov., no hypothesis can be proposed as long as the semiterrestrial and cave fauna of the Macaronesian islands and the groundwater copepods of North Africa (Morocco) are unknown, and a well-resolved, robust phylogeny of the genus *Bryocyclops* is lacking.

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