RESEARCH ARTICLE



# New oversize troglobitic species of Campodeidae in Japan (Diplura)

Alberto Sendra<sup>1,2</sup>, Kazunori Yoshizawa<sup>3</sup>, Rodrigo Lopes Ferreira<sup>4</sup>

 Grupo de Investigación de Biología del Suelo y de los Ecosistemas Subterráneos, Departamento de Ciencias de la Vida, Facultad de Biología, Ciencias Ambientales y Química, Universidad de Alcalá, E-28871, Alcalá de Henares, Madrid, Spain 2 Servei de Patrimoni Històric, Ajuntament de Valencia, E-46008, Valencia, Spain
Systematic Entomology, School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan 4 Centro de Estudos em Biologia Subterrânea, Setor de Biodiversidade Subterrânea – Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil

Corresponding author: Alberto Sendra (alberto.sendra@uv.es)

Academic editor: Oana Moldovan   Received 23 July 2018   Accepted 7 October 2018   Published 23 October 20	018
http://zoobank.org/6AB65E0F-8BDB-45CC-B880-A295B3EC3E1B	

**Citation:** Sendra A, Yoshizawa K, Ferreira RL (2018) New oversize troglobitic species of Campodeidae in Japan (Diplura). Subterranean Biology 27: 53–73. https://doi.org/10.3897/subtbiol.27.28575

#### Abstract

Two new oversize troglobitic species of diplurans campodeids, Pacificampa daidarabotchi Sendra, sp. n. and Pacificampa nipponica Sendra, sp. n., found in three caves in two southern Japanese islands are described. It is the first record of cave-dwelling Diplurans from Japan and more specifically these are two Campodeinae of Pacificampa Chevrizov, 1978 formally known in continental Asia with three cave-dwelling species described in Russia and China near the north of the Korean peninsula. In P. daidarabotchi sp. n., in addition to its oversized body, the longest ever known in campodeids family, it shows as its main differential taxonomical feature the absence of lp metanotal macrosetae. P. nipponica sp. n. is much closer to continental Pacificampa species but it shows differences in the urotergal macrosetae formula. Both Japanese species have clear troglomorphic features related with their elongated body and appendages and an increase in number and complexity of the sensorial antennal equipment with unique olfactory chemoreceptors. Two biogeographical remarks are inferred: P. nipponica sp. n., has been found in two islands that were connected during the glacial age and *P. daidarabotchi* sp. n. lives in a single cave near another where P. nipponica sp. n. dwells which allowing to suggest a sympatric distribution; Pacificampa seems to be related with *Eumesocampa*, a genus from North-America, as a consequence of the Holarctic connection between Asia and America. The interests of P. daidarabotchi sp. n. and P. nipponica sp. n. as endemic species in addition to their biogeographical importance should be taken into consideration to ensure better management of the three touristic caves where they dwell and especially the protection of Mejido-do cave, the only location of the large P. daidarabotchi sp. n. near to an active quarry.

Copyright Alberto Sendra et al. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

#### **Keywords**

Kyushu Island, Shikoku Island, Pacificampa daidarabotchi, Pacificampa nipponica, cave fauna, troglobite, subterranean , biogeography

## Introduction

To date no data is available about subterranean dipluran fauna in Japanese caves or any subterranean habitats. The knowledge of diplurans in Japan is limited to soil habitats, the first one was a japygid described by Enderlein (1907). About twenty years later Kuwayama (1928) and Silvestri (1928) added seven species for Japanese islands including two Parajapygidae ones. In 1931 Silvestri described five species of Campodeidae, four within Campodeinae and one within Lepidocampinae, most endemic to Japan.

No species of Diplura have described from that moment on, until when an expedition to caves in Japan was carried out from September 5 to 15, 2017. The main objective of this expedition was to sample cave invertebrates in order to understand how communities respond to habitat traits (manuscript in prep.). Eleven carbonate caves were sampled (three on Shikoku Island and eight on Kyushu Island). In Shikoku island, the following caves were sampled: Hiura-do cave (Kumakogen municipality), Inaba-do cave (Tsuno municipality) and Rakan Ana cave (Seiyo municipality). In Kyushu island, the following caves were sampled: Goya Daini Shonyu-do, Goya Daiichi Shonyu-do, Merijo-do and Goya Daysan-do caves (all located in Kitakyushu municipality); Nichiko-do and Nichiko Ni-do caves (both located in Yatsushiro municipality) and Takazawa Shonyu do and Osê-do caves (both located in Kuma municipality) (Figs 25–39).

#### Material and methods

#### Sampling methods

The fauna collections were carried out both in transects and throughout several habitats of the caves, prioritizing areas (in the case of general collections) with accumulations of organic matter. The collections were done manually, using brushes and tweezers. The organisms were placed in 70% ethanol immediately after collection. Diplura specimens were found in only three caves (Mejiro-do, Goya Daiichi Shonyu-do and Inaba-do caves) in Kyushu and Shikoku Islands.

#### Material processing and identification

The specimens were washed using distilled water and were mounted on slides with Marc André II medium to be examined under a phase-contrast optical microscope (Leica DMLS). The illustrations were made with a drawing tube, and the measurements were taken with a micrometer ocular. To take measurements of body length, the specimens were mounted "in toto" and measured from the base of the frontal process distal macrochaetae to the abdomen's supra-anal valve. For scanning electron microscopy (Hitachi S-4100), two paratypes were coated with palladium-gold used for scanning electronic microscopic photography and measurement of the sensilla.

The morphological descriptions and abbreviations used in this paper follow Condé (1956). We use gouge sensilla for the concavo-convexly shaped sensilla located on the antennae and described by Bareth and Condé (1981), whose function is still unknown. For the position of macrosetae we follow Condé (1956): *ma*, medial-anterior macrosetae; *la*, lateral-anterior macrosetae; *lp*, lateral-posterior macrosetae; *sma*, medial-anterior submacrosetae:

#### Results

#### Taxonomy

#### Pacificampa daidarabotchi Sendra, sp. n.

http://zoobank.org/106F3EAB-000B-4329-8028-98D87A35FD4C Figs 1–22, 31, Tables 1, 2

**Etymology.** This species is the largest Campodeidae known to date. Daidarabotchi is a giant in Japanese mythology.

**Type material.** Female holotype labeled ME02 from Mejiro-do Cave (33.763N, 130.907E), ISLA 47548, Japan, 10 September 2017, Ferreira, R.L. leg. (SEHU); 1  $\stackrel{\circ}{\supset}$  labeled ME01 (AS), 1 young female labeled ME03 (SEHU) paratypes from the same type locality, data and leg. mounted in Marc André solution. And 1  $\stackrel{\circ}{\supset}$ , 1  $\stackrel{\circ}{\ominus}$  paratypes from the same type locality, data and leg mounted in separated aluminum stages and coated with palladium-gold (AS). Deposited AS collection and Hokkaido University Insect Collection: SEHU.

**Description.** Body length 9.5 mm (male) paratype ME01), 10.1 mm (female, holotype) and 6.5 mm (young paratype ME03) (Fig. 31). Epicuticle smooth under optical microscope but reticulated in high magnifications (Figs 8–10); body with abundant thin and smooth clothing (Fig. 17).

One intact antenna in the male paratype ME01 with 37 antennomeres, slightly (1.07) longer than the body (the other antennae of the same specimen is presumably regenerated with 28 antennomeres, 7.4 mm length) and another intact antenna in the young female ME03 with 41 antennomeres, 1.21 times longer than the body (Table 1). Apical antennae 4.4–4.7 longer than wide and central antennomeres 2.5 longer than wide Fig. 1). Cupuliform organ occupying 1/11 of the total length of the apical antennomere with 8–11 unique olfactory chemoreceptors each one with a complete caliciform pocket that encloses complex folds in radial expansions coming from a central axis, both of which are covered by small pores (Figs 2, 3). Distal and central antennomeres



**Figures 1–6.** *Pacificampa daidarabotchi* Sendra, sp. n. **1** Last and penultimate antennomere, paratype ME01 **2** olfactory chemoreceptors within the cupuliform organ, paratype **3** detail olfactory chemoreceptor, paratype **4** coniform sensilla on the antennomeres, paratype **5** gouge sensilla on distal whorl on distal antennomere, paratype **6** detail gouge sensillum, paratype.



**Figures 7–10.** *Pacificampa daidarabotchi* Sendra, sp. n. **7** Pro-, meso- and metanotum, right side, holotype **8** detail epicuticle surface on mesonotum, paratype **9** detail epicuticle surface including external gland in the middle on mesonotum, paratype **10** detail epicuticle surface and the ecdysial suture on metanotum, paratype.



**Figures 11–15.** *Pacificampa daidarabotchi* Sendra, sp. n. **11** Metathoracic leg, paratype ME01 **12** calcars, paratype **13** tibial portion, lateral side, paratype **14** detail claws, dorsal-lateral side, paratype **15** ending telotarsal leg, lateral-anterior side, paratype.

Specimen	Body	antennomeres	Antennae	Seg	ment length o	Total			
	length		length	Coxa	Trochanter	Femur	Tibia	Tarsus	length
paratype ME03	6.5	41	7.9	0.30	0.35	1.05	1.10	1.00	3.8
paratype ME01	9.5	37	10.2	0.40	0.35	1.35	1.55	1.35	5.0
holotype ME02	10.1	_	_	0.40	0.40	1.58	1.63	1.40	5.4

**Table 1.** *Pacificampa daidarabotchi* Sendra, sp. n., length of the body, antennomeres and metathoracic leg including their segments, and cerci (units in mm); and, number of antennomeres.

with a sensorial equipment of macrosetae and setae in addition to a single distal whorl of 12–16 thin and long gouge sensilla (Figs 5, 6) 42–34  $\mu$ m long and a 2–3 very short coniform sensilla 8  $\mu$ m long, also present in the apical antennomere (Fig. 4). Proximal antennomeres with typical trichobothria plus the sensillum of the third antennomere coniform and slightly thick located in ventral position between *d* and *e* macrosetae.

Plain frontal process with the three frontal macrosetae smooth. The three macrosetae along each side of the line of insertion of antennomere and *x* setae smooth and with 31/51/21/30 (*a/i/p/x*) relatives lengths. Suboval labial palps with latero-external short thick sensillum, with two guard setae, up to 12 setae on anterior border and up to 130 neuroglandular setae.

Thoracic macrosetae distribution (Fig. 7): pronotum with 1+1 ma, 1+1 la, 1+1 lp; mesonotum with 1+1 ma, 1+1 la, 2+2  $l_{p_2}$  (1+2  $l_{p_3}$ - $l_{p_2}$  in paratype ME01); metanotum with 1+1 sma, 1+0 ma in paratype ME01) macrosetae. All macrosetae relatively well developed but *lp* slightly thicker and longer than the others, and all with thin short barbs along the distal third or fourth; marginal setae longer thicker than clothing setae, smooth or with a few tiny distal barbs. Legs elongated, metathoracic legs reaching the IX abdominal in the adults (ME01 and ME02) and the end of the abdomen in the young female (paratype ME03) (Table 1). Femur I–III with one dorsal macroseta (Fig. 11) well differentiated but smooth. Calcars slightly differentiated with a few thin apical barbs (Fig. 12). The tibial two rows of ventral setae are longer and thicker than clothing setae but smooth like the clothing setae (Fig. 13). Tibia I-II with one ventral macroseta, tibia III with two ventral macrosetae (1+2 in paratypes ME01 and ME03) with a few tiny thin apical barbs. Dorsal and lateral tarsal setae similar to clothing setae but much longer (Fig. 15). Slightly unequal to subequal slightly curved claws (posterior claw 1.05–1.25 longer than anterior one); dorsal side of the claws have more pronounced ridges than the ventral side (Figs 14); any expansion of the claws is present.

Distribution of abdominal macrosetae on tergites (Fig. 16): 1+1 *sma* on V (one unilateral *ma* in holotype; in addition to 0+1 *la* and 0+1 *lp*<sub>3</sub> in paratype ME01); 1+1 *ma*, 1+1 *la* and 1+1 *lp*<sub>3</sub> on VI (0+1 *sla* and 1+0 *la* in holotype and 2+2 *lp*<sub>2,3</sub> in paratype ME01); 1+1 *ma*, 1+1 *la* and 1+1 *lp*<sub>3</sub> on VII (2 *lp*<sub>2,3</sub>+1 *lp*<sub>3</sub> in holotype and 2+2 *lp*<sub>2,3</sub> in paratype ME01): 1+1 *mp*, 1+1 *slp*<sub>1</sub>, 2+2 *lp*<sub>2,3</sub> on VIII; 5+5 *lp*<sub>1,2,3,4,5</sub> on IX abdominal segment (5 *lp*<sub>1-5</sub>+6 *lp*<sub>1-6</sub> in holotype). All tergal abdominal macrosetae long and well differentiated with thin barbs along the distal third; submacrosetae thinner and shorter (in particularly the *slp*<sub>1</sub> of the VIII urotergite) and smoother than macrosetae.



Figures 16–17. *Pacificampa daidarabotchi Sendra*, sp. n. 16 Urotergites V–IX, left side., paratype ME01 17 detail urostergite VIII, paratype.



**Figures 18–22.** *Pacificampa daidarabotchi* Sendra, sp. n. **18** Urosternite I, *S* paratype ME01 **19** Urosternite VII (left side) plus urosternite VIII, *S* paratype **20** Stylus urosternite II, paratype **21** Urosternite VI, left side, *Q* paratype **22** Urosternite VIII, *Q* paratype.

Urosternite I with 7+7 macrosetae (Fig. 18); urosternites II to VII with 4+4 macrosetae; urosternite VIII with 1+1 macrosetae; almost all urosternal macrosetae long, well differentiated and covered by long barbs along the distal half (Figs 19–22). Elongated styli with smooth apical setae of styli with a short tooth with one tiny thin barb; also, smooth subapical and ventromedial setae being the ventromedial much longer than the others (Fig. 20). Cerci in the holotype with 9 and 14 articles including the basal article, slightly longer than the body length (1,04–1,12); in paratype young female ME03 has a cercus with six articles and 1.25 times longer than the body length; their

	Cerci	Articles length												<b>77</b> 1		
Specimen	Articles and position	Basal	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>	11 <sup>th</sup>	12 <sup>th</sup>	13 <sup>th</sup>	lotal length
paratype ME03	6 right	1.7	0.7	1.1	1.4	1.6	1.6									8.1
Holotype	9 right	1.9	0.65	0.85	0.9	1.05	1.15	1.27	1.3	1.4						10.47
ME02	14 left	1.65	0.45	0.50	0.55	0.55	0,55	0.60	0.70	0.75	0.90	0.95	1.0	1.0	1.2	11.35

**Table 2.** *Pacificampa daidarabotchi* Sendra, sp. n., cercal articles length and total length (units in mm) including number of articles of each cerci.

basal articles show several whorls of long thin macrosetae with a few apical barbs, along the medial and distal articles these long macrosetae became smooth and whorls of shorter smooth setae are present among them; all the articles are characterized by an apical whorl of thin smooth setae also present at the end of the last article (Table 2).

Female urosternite I with subcylindrical appendages thinner than male appendages (2,7 times longer than wide), each bearing up to 55  $a_1$ -glandular setae in a distal field.

Male urosternite I (Fig. 18) with moderated thick subcylindrical appendages (2 times longer than wide), each bearing up to 90  $a_1$ -glandular setae in a large field covering almost a distal third of the appendage.

Spermatozoid fascicles present in the paratype male testis but difficult to observe. They are about 100  $\mu$ m in diameter with a spiral round structure up to 4–7  $\mu$ m in diameter with 3–5 turns.

**Remarks.** Although the former description of *Pacificampa* Chevrizov, 1978 in Russia near the north of the Korean peninsula had a poorly drawn text, the differential features of the genus were clear: simple subequal claws with no lateral telotarsal process, 3+3 (*ma, la, lp*) pronotum, 4+4 (*ma, la, lp*<sub>2,3</sub>) on mesonotum and 2+2 (*ma, lp*<sub>2</sub>) on metanotum with the exception of 1+1 *ma* in *P. daidarabotchi* sp. n.; one dorsal femoral macroseta and two ventral tibial macrosetae. Nevertheless, no description of the sexual features was cited in the diagnosis of the genus, nor in any of the proposed species (*Pacificampa birsteini* Chevrizov, 1978 and *Pacificampa caesa* Chevrizov, 1978), perhaps this was due to their simplicity. In both sexes the first urosternal appendages are subcylindrical with distal *a1* glandular setae slightly more abundant in males than in females. No other glandular setae are present in the first urosternite.

*P. daidarabotchi* sp. n. differs from other *Pacificampa* species in several features including its long body with 10.1 millimeters, that is the longest Campodeidae that has ever been recorded. An oversize body could be the rule in the rest of *Pacificampa* species although there is still not enough data to demonstrate this. Furthermore, it has longer antennae and cerci and more numerous antennomeres and cercal articles than other species of the genus; although the most notable features are the reduction of metanotal macrosetae with  $1+1 ma (1+1 ma, 1+1 lp_3)$  in others species of the genus) and the reduction and variability in number of urotergal macrosetae.

http://zoobank.org/5B8F1DCC-CC0A-4A28-A147-D4754CF69ECA Figs 23, 24, 36, 39, Tables 3–4

#### Etymology. Species epithet refers to Japan.

**Type material.** Male holotype labeled SH01 from Goya Daiichi Shonyu-do Cave (33.689N, 130.811E), ISLA 47550, Japan, 9 September 2017, Ferreira, R.L. leg. (SEHU); 1 juvenile paratype labeled from the same type locality (AS), data and leg. mounted in Marc André solution. Deposited AS collection and Hokkaido University Insect Collection: SEHU

Other studied material. 1  $\Diamond$ , 1  $\bigcirc$  labeled IN01 and IN02 from Inaba-do Cave (33.439N, 133.086E), ISLA 47549, Japan, 7 September 2017, Ferreira, R.L. leg. (AS).

**Description.** Body length 5.0 and 6.3 mm (males), 6.0 mm (female) and 4.8 mm (juvenile) (Fig. 36–39, Table 3). Epicuticle smooth under optical microscope; body with abundant thin and smooth clothing.

One intact antenna in the male holotype SH01 with 34 antennomeres, longer (1.17) than the body. Apical antennae 4.6 times longer than wide and central antennomeres 3.5 times longer than wide (Table 3). Cupuliform organ occupying 1/10 of the total length of apical antennomere about 8 complex olfactory chemoreceptors. Distal and central antennomeres with a sensorial equipment of macrosetae and setae in addition to a single distal whorl of 12–14 thin and long gouge sensilla and 2–3 very short coniform sensilla, also present in the apical antennomere. Proximal antennomeres with typical trichobothria plus the sensillum of the third antennomere coniform located in dorsal position between b and c macrosetae.

Plain frontal process with the three frontal macrosetae smooth. The three macrosetae along each side of the line of insertion of antennomere and *x* setae smooth and with 25/40/26/20 (*a/i/p/x*) relative lengths. Suboval labial palps with latero-external short thick sensillum, with two guard setae, up to seven setae on anterior border and up to 110 neuroglandular setae.

Thoracic macrosetae distribution (Fig. 23): pronotum with 1+1 ma, 1+1 la, 1+1 lp; mesonotum with 1+1 ma, 1+1 la, 2+2 lp<sub>2,3</sub>; metanotum with 1+1 ma, 1+1 lp<sub>3</sub> macrosetae. All macrosetae developed slightly thick and with short barbs along the distal half or third; marginal setae longer and thicker than clothing setae with distal thin barbs. Legs elongated, metathoracic legs reaching the IX abdominal in the adults (ME01 and ME02) and the IX abdominal in the juvenile (paratype SH02) (Table 3). Femur I–III with one long dorsal macrosetae well differentiated with a few distal barbs. Tibia I–II with one ventral macroseta, tibia III with two ventral macrosetae with a few tiny thin apical barbs. Calcars differentiated with 1–3 long distal barbs. The tibial two rows of ventral setae are longer and thicker than clothing setae but smooth like the clothing setae. Dorsal and lateral tarsal setae similar to clothing setae but much longer (Table 3). Subequal slightly curved plain claws (posterior claw 1.05 longer than anterior one); no expansion of the claws is present.

Distribution of abdominal macrosetae on tergites (Fig. 24): 1+0 *sma* on II (only in the juvenile SH02); 1+1 *sma* on III (only in the juvenile SH02); 1+1 *ma* (0+1 *lp* in



Figures 23–24. *Pacificampa nipponica* Sendra, sp. n. 23 Pro-, meso- and metanotum, right side 24 Urotergites IV–IX, right side.



**Figure 25.** Map of Japan highlighting Kyushu (**A**) and Shikoku (**B**) Islands. The red star indicates the location of Mejiro-do Cave (habitat of *P. daidarabotchi*), while the yellow stars indicate the location of Goya Daiichi Shonyu-do (Kyushu) and Inaba-do (Shikoku) caves, both habitats of *P. nipponica*.

	Body		Antonnao	Seg	Total				
Specimen	length	antennomeres	length	Coxa	Trochanter	Femur	Tibia	Tarsus	metathoracic length
paratype SH02	4.8	-	-	0.20	0.17	0.60	0.70	0.65	2.32
IN01	5.0	-	-	0.30	0.25	0.75	0.80	0.75	2.85
IN02	6.0	-	-	0.30	0.30	0.83	0.87	0.81	3.11
Holotype SH01	6.3	34	7.4	0.30	0.30	0.91	1.0	0.86	3.37

**Table 3.** *Pacificampa nipponica* Sendra, sp. n., length of the body, antennomeres and metathoracic leg including their segments, and cerci (units in mm); and, number of antennomeres.

juvenile SH02 and male IN01 and 0+1 *sla* in female IN02) on IV; 1+1 *ma*, 1+1 *la* and 2+2  $lp_{2,3}$  on V-VII; 1+1 *mp*, 3+3  $lp_{1,2,3}$  on VIII; 1+1 *mp*, 5  $lp_{1,2,3,4,5}$  on IX abdominal segment. All tergal abdominal macrosetae thick, long and well differentiated with thin barbs along the distal half to third; submacrosetae thinner and shorter than macrosetae and smooth as macrosetae.

Urosternite I with 6+6 macrosetae (5+5 in juvenile SH02); urosternites II to VII with 4+4 macrosetae; urosternite VIII with 1+1 macrosetae; almost all urosternal macrosetae long, well differentiated and covered by long barbs along the distal half. Smooth apical setae of styli with a short tooth with one tiny thin barb; also, smooth subapical and ventromedial setae, the ventromedial being much longer than the others. Cerci in the holotype with six articles including the basal article, slightly shorter than the body length (0.99); in juvenile paratype young SH02 has two cerci with six and seven articles, 1.30 and 1.41 longer than the body length; their basal articles show several whorls of long thin macrosetae with a few apical barbs, along the medial and distal articles these long macrosetae became smooth and whorls of sorther smooth setae are present



**Figures 26–31. 26** Karst landscape in the area where Mejiro-do cave is located **27** Map of Mejiro-do cave, indicating the touristic area (marked in pink), and the locations where specimens of *P. daidarabotchi* were found (red dots) **28** conduit in the non-touristic area, trespassed by a stream **29** touristic area near the cave entrance **30** speleothems, where some specimens can be observed 31 *P. daidarabotchi*, living specimen.



**Figure 32.** Karst landscape in the area where Mejiro-do cave is located (yellow star). Below, the quarry, whose limits are less than 2km-from the Mejiro-do cave entrance.

among them; all the articles are characterized by an apical whorl of thin smooth setae also present at the end of the last article (Table 4).

Female urosternite I with subcylindrical appendages slightly thinner than male appendages 2.2 times longer than wide, each bearing  $30 a_1$ -glandular setae in a distal field.

Male urosternite I with moderated thick subcylindrical appendages 2 times longer than wide, each bearing up to 50  $a_1$ -glandular setae in a field covering the distal fifth of the appendage.



**Figure 33–39. 33** Karst landscape in the area where Inaba-do cave is located **34** Inaba-do cave entrance **35** chamber located at the innermost area of Inaba-do cave **36** *P. nipponica*, living specimen from Inaba-do cave **37** stone wall built in Goya Daiichi Shonyu-do cave **38** mettalic stairs in Goya Daiichi Shonyu-do cave **39** *P. nipponica*, living specimen from Goya Daiichi Shonyu-do cave.

<b>C</b>			Teastlansah						
Specimen	Articles and position	Basal	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	$4^{\text{th}}$	5 <sup>th</sup>	6 <sup>th</sup>	Iotai length
Paratype SH02	6 left	1.30	0.60	0.75	1.05	1.30	1.25		6.25
	7 right	2.65	0.50	0.55	0,.0	0.70	0.80	0.95	6.75
Holotype SH01	6 (?)	1.40	0.60	0.80	1,00	1.20	1.25		6.25

**Table 4.** *Pacificampa nipponica* Sendra, sp. n., cercal articles length and total length (units in mm) including number of articles of each cerci.

**Remarks.** *P. nipponica* Sendra sp. n. is closer to the continental *Pacificampa* (*P. birsteini, P. caesa* by Chevrizov (1978) and the not-formally-described *Pacificampa* species by Ferguson (1997) from China, northern Korean peninsula) with some taxonomic differences in: presence of 1+1 *ma* macrosetae on urosternite IV, and urosternites II to VII with 4+4 macrosetae (instead of 5+5).

#### Discussion

#### Habitat and conservation issues

*P. daidarabotchi* Sendra sp.n. was found in Mejiro-do Cave (33.763N, 130.907E) which is used for touristic purposes. This limestone cave is located in Kitakyushu City (Fukuoka Prefecture) on Kyushu Island (Fig 25A). The external environment is a typical karst landscape (Fig. 26) and is currently protected, although severe impacts occur in the surroundings. The cave entrance, as well as its first 200 meters, were adapted to the tourism, through the installation of electric lighting, pathways, stairs and banisters (Figs 27, 29). This area is severely altered, and only invertebrates typically found near entrances (such as crickets and moths, for example) are observed. However, tourists do not access most parts of the cave, which presents ca. 2,160 m of horizontal projection. The lower level (comprising the biggest part of the cave), is trespassed by a drainage system (Fig. 28), which brings in plant debris from the external environment, constituting the main organic resource available for the subterranean fauna.

Individuals of *P. daidarabotchi* Sendra sp.n were only found in deeper areas of the cave (at least 250 m far from the entrance), which are relatively well preserved (Fig. 28). Specimens were found freely walking on the muddy sediments surrounding the stream in the main conduit of the cave, over calcite deposits (speleothems – Fig. 30) and sometimes under rocks (Fig. 31). Other troglobitic species found in this cave include millipedes, spiders, springtails and beetles (*Stygiotrechus*). However, although the cave is located within a protected area, an extensive quarry is operating in the surroundings (Fig. 32), which brings some concern regarding the protection of this species. In a straight line, the cave entrance is located less than two kms from the boundary of the quarry. Accordingly,

it is advisable to start a monitoring of the cave fauna, in order to assess alterations in the communities or populations, with respect to the expansion of the quarry.

Inaba-do Cave (33.439N, 133.086E) comprises a limestone cave associated to the Chichibu zone, a Jurassic accretionary prism. It is located in Tsuno Town (Kochi Prefecture), in Shikoku Island (Fig. 25B). The single entrance is located at the base of a limestone mountain and is used for touristic and religious purposes (Figs 33-34). The cave entrance region was modified to facilitate the access of humans, so that stairs and pathways were built, thus altering its pristine conditions. A stream appears (in an upwelling) in a conduit not far from the entrance, trespassing part of the cave. Although the cave is touristic, some passages are considerably narrow, thus preventing the access of visitors to the innermost portions of the cave, which are well preserved. In the deepest part of the cave there is a big chamber, formed by the collapse of rocks from the walls and ceiling, although most of the blocks are covered by a clay layer (Fig. 35). Along the conduits (especially those trespassed by the stream), there are banks of sediments, where the specimens of *P. nipponica* Sendra sp.n were found (Fig, 36). They were freely walking on this sediment, apparently looking for trophic resources. Although there are bats in the cave, their colonies were not big, so that guano probably does not constitute the main resource in this cave. Plant debris brought by water from external environments seems to constitute a more regular food supply for the cave fauna. Other troglobitc invertebrates observed in this cave include springtails and carabidae beetles (Ishikawatrechus cerberus).

*Pacificampa nipponica* Sendra sp. n. was also found in Goya Daiichi Shonyu-do cave (33.689N, 130.811E), another show cave located in the same municipality of Mejiro-do cave (in Kyushu Island – Fig. 25B). It presents around 180 m of horizontal projection, but contrary to what is observed in Mejiro-do cave, practically all its extension is used for tourism. Thus, virtually the entire length of this cave is quite altered, existing pathways, stairs (Fig. 38), gates and containment structures (stone walls - Fig. 37) that severely altered the pristine conditions of the cave. The trophic resources observed consisted mainly of bat guano. Specimens were observed in the final portion of the cave, under rocks present in the floor, in places further away from the areas directly trampled by tourists (Fig. 39). Considering that this cave presents uncontrolled tourism, it would be also advisable to monitor its invertebrate communities, in order to verify the effect of the tourism on the different species.

# Phylogenetic affinities of the genus *Pacificampa* with notable biospeleological and biogeographical remarks

The characteristics of 3+3 (*ma*, *la*, *lp*) pronotal macrosetae and the simple claws without lateral crests put *Pacificampa* into Campodeinae, a subfamily widespread in all continents except in Antarctica (Conde 1956). The absence of lateral telotarsal process and the extra macrosetae on metanotum, with 2+2  $lp_{2,3}$  are not common in Campodeinae and this combination characterize *Pacificampa* genus, distinguishing it from other Campodeinae genera (Chevrizov 1978). These uncommon features are independently present in a few genera, such as *Afrocampa* Silvestri, 1933, *Anisocampa* Silvestri, 1932, *Eumesocampa* Silvestri, 1933, *Mexicampa* Wygodzinsky, 1944, *Natalocampa* Condé, 1951 and *Metriocampa* Silvestri, 1912. These two distinctive features suggest some affinities mainly with the closely distributed genera, *Eumesocampa* and *Metriocampa*. These close affinities between *Eumesocampa* and *Pacificampa* were previously suggested by Ferguson (1997).

*Eumesocampa*, with three soil-dwelling species cited in several localities in the United States of North-America (Allen 1994), has a similar notal macrosetae pattern to *Pacificampa*, but claws show a thin spiniform lateral expansion. Furthermore, Ferguson (1982, 1989) quoted two new undescribed species of *Eumesocampa* in several caves in the mid-western States.

*Metriocampa*, is more widespread than other genera, with 16 already described species (Allen 1994, Ferguson 1997, Chou and Chen 1980) and although in some species share the same telotarsus structure as *Pacificampa*, all species of *Metriocampa* do not have extra macrosetae in mesonotum nor metanotum. They have been found in endogean habitats from North-American localities to the east of Russia, China (Chou and Chen 1980) and in Japan (Silvestri 1931) and the most northern location of any dipluran, in Alaska (Sikes and Allen 2016). These three related genera, *Eumesocampa, Metriocampa* and *Pacificampa*, show former connection of East Asia and North-America and a result nowadays of a vicariance distribution at subfamily and genus level.

All five known *Pacificampa* species have been found in caves. *P. birsteini, P. caesa,* including one undescribed *Pacificampa* species from China (Ferguson 1997) are known of in three different caves in continental karstic areas; in the extreme east of Russia and in the north-east of China, all in the north of the Korean peninsula. The two new species were collected in two southern islands of Japan, Shikoku and Kyushu Islands. In the case of continental species, they have apparently no clear troglomorphic features perhaps because of the poor description of the specimens. On the contrary, *P. nipponica* Sendra n. sp. and *P. daidarabotchi* Sendra n. sp. show some troglomorphic features, more evident in *P. daidarabotchi* n. sp. than in *P. nipponica* n. sp. such as: abundant and complex sensilla of the cupuliform organ; large body with all appendages moderately elongated (Tables 1–4), elongation that is clear in antennomeres and cercal articles; and, an increase in the number of antennomeres and cercal articles compared to other soil-dwelling Campodeinae species and the cave-dwelling continental species of *Pacificampa*.

The presence of *P. nipponica* Sendra sp. n. in two caves on Shikoku Island and Kyushu Islands is remarkable. These two islands are now separated by a sea, but it is known that they were connected during the last glacial age (20,000–10,000 years ago). Another exemple, among the cave-dwelling fauna, that highlights the biogeographical affinity of these two islands, is that related to a couple of cryptic troglobitic beetle species of the subgenera *Paratrechiama* and *Pilosotrechiama* of the genus *Rakantrechus* (Trechinae) (Uéno and Naitô 2008).

*P. daidarabotchi* sp. n. was collected in Mejiro-do Cave, Kyushu Island, nearby the cave from which *P. nipponica* sp. n. was collected. A similar distribution, also dwelling

in the same cave, has been reported in several subterranean campodeids; *Campodea* (*Campodea*) grassii Silvestri, 1912 and *Campodea* (*Campodea*) majorica Condé, 1955 from the extreme east of the Iberian peninsula (Sendra and Moreno 2004; D0528); *Podocampa asturiana* Sendra, Monedero & Salgado, 2003 and *Podocampa simonini* Condé, 1956 in the Cantabrian and Basque Mountains in the north of the Iberian Peninsula (Sendra et al. 2003; Sendra et al. in press) and *Plusiocampa (Plusiocampa)* balsani Condé, 1947 and *Plusiocampa (Plusiocampa) dargilani* (Moniez, 1894) in the Grands Causses south of Massif Central (Condé 1997). In such pairs of species with a sympatric distribution, the first one occupies a larger range than the second species, and the second goes much further in the troglomorphic process adaptation to the subterranean ecosystem. A remarkable fact that ought to be explored in the future.

#### Acknowledgements

Firstly, we would thank Marconi Souza Silva, T. Komatsu and Y. Hara for bringing up the vital sampling effort made. For T. Yamashita, the owner of Mejiro Cave, for allowing us to access the cave. The field trip in Japan was supported by JSPS research grant 15H04409 to KY. Investigation of Mejiro Cave was conducted under the permission from Fukuoka Prefecture (permission ID 29-628). We are also indebted to the electron microscopy facility at the Universitat de València, specially with Enrique Navarro, Pilar Gómez and Rafael Benito for their help and instructions on preparing the material for the scanning electron microscope and obtaining the photographs. We also thank Katie Marsen for helping us translate this paper. We would like also to thanks the revisions works made by Loris Galli and Alessandro Giupponi. R. L. Ferreira is grateful to the National Council of Technological and Scientific Development (CNPq) for a research grant (Process number 304682/2014-4).

### References

- Allen RT (1994) An annotated checklist and distribution records of the subfamily Campodeinae in North America (Insecta: Diplura: Rhabdura: Campodeidae). Transactions of the American Entomological Society 120 (3): 181–208.
- Bareth C, Condé B (1981) Nouveaux Campodéidés de grottes d'Espagne. Revue suisse Zoologie 88 (3): 775–786. https://doi.org/10.5962/bhl.part.82407
- Chevrizov BP (1978) Two new genera of the Family Campodeidae from the Far East Caves. Zoologichesky Zhurnal 57(2): 197–205.
- Chou I, Chen T (1980) Two new species of campodeidae from Xinjiang (Apterygota : Diplura). Entomotaxonomia 2(2): 157–160.
- Condé B (1956) Matériaux pour une Monographie des Diploures Campodéidés. Mémoires du Muséum National d'Histoire naturelle Série A. Zoologie 12: 1–202.

- Condé B (1962) Géonémie des Diploures troglobies du Jura et du Vercors. Spelunca Mémoires 2: 119–127.
- Condé B (1997) Campodéidés des grottes du Parc Naturel Régional des Grands Caussess (Insecta, Diplura). Mémoires de Biospéologie 24: 157–162.
- Enderlein G (1907) Über die Segmental-Apotome der Insekten und Kenntnis der Morphologie der Japygiden. Zoologischen Anzeiger 31 (19/20): 629–635.
- Ferguson LM (1982) Cave Diplura of the United States. Proceedings of the Eighth International Congress Speleology Kentuky 1928: 11–12.
- Ferguson LM (1989) Taxonomy and Distribution of the *Eumesocampa* (Diplura: Campodeidae) of North America. Proceedings of the 10<sup>th</sup> International Congress of Speleology Budapest 2, 680.
- Ferguson LM (1997) A report on a new species of *Pacificampa* (Diplura: Campodeidae) from a cave in China and a comparison of some North America genera to *Pacificampa* and *Plutocampa* previously only known from the Far East of Russia. Proceedings of the 12<sup>th</sup> International Congress of Speleology La Chaux-de-Fonds, Neuchâtel, Switzerland, August 10–17, 3, 315–317.
- Kuwayama S (1928) Some Japanese species of Japyx. Insecta Matsumurana 2: 151–155.
- Sendra A, Moreno A (2004) El subgénero *Campodea* s.str. en la Península Ibérica (Hexapoda: Diplura: Campodeidae). Boletin Sociedad Entomológica Aragonesa 35: 19–38.
- Sendra A, Salgado JM, Monedero E (2003) Dos nuevas especies y una subespecie de campodeidos cavernícolas de la cornisa cantábrica (Diplura Campodeidae). Animal Biodiversity and Conservation 26.2: 69–80.
- Sikes DS, Allen RT (2016) First Alaskan records and a significant northern range extension for two species of Diplura (Diplura, Campodeidae). ZooKeys 563: 147–157. https://doi. org/10.3897/zookeys.563.6404
- Silvestri F (1928) Japygidae (Thysanura) dell'Estremo Oriente. Bolletino del Laboratorio di Zoologia generale e agraria in Portici 22: 49–80.
- Silvestri F (1931) Campodeidae (Insecta Thysanura) dell'Estremo Oriente. Bollettino del Laboratorio di Zoologia generale ed agraria del R. Istituto superiore agraria di Portici 25: 286–320.
- Uéno S, Naitô T (2008) Occurrence of Shikoku representative of blind trechines (Coleoptera, Trechinae) originated in Eastern Kyushu, southwest Japan. Elytra 36: 213–225.