A revision and phylogenetic analysis of the millipede genus

**Oxidus** Cook, 1911 (Polydesmida, Paradoxosomatidae)

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The genus **Oxidus** Cook, 1911 is revised to contain five species, *O. avia* (Verhoeff, 1937), *O. gigas* (Attems, 1953), *O. gracilis* (C.L. Koch, 1847), *O. riukiaria* (Verhoeff, 1940), and “species inquirenda” *O. obtusus* (Takakuwa, 1942). A cosmopolitan species, *O. gracilis*, is widely found in temperate and sub-tropical regions over the world, but other species have limited distribution in restricted regions, e.g., *O. gigas* in northern Vietnam, *O. riukiaria* and *O. avia* in the Ryukyu Islands (Japan). Four species, *O. gracilis*, *O. riukiaria*, *O. avia* and *O. gigas*, are confirmed as different from each other in gonopod characters, coloration and body size. The status of the last species, *O. obtusus*, is still doubtful and requires examination of further fresh material. The phylogenetic relationships among species of *Oxidus* is analyzed using two fragments of the mitochondrial genes COI (Cytochrome c Oxidase subunit I) and 16S rRNA. Three species of *Oxidus* are clearly separated from each other; *O. gigas* and *O. gracilis* form a monophyletic sister group with *O. riukiaria*. The genus *Oxidus* is also monophyletic and more closely related to the genus *Tylopus* Jeekel, 1968 than to the genera *Sellanucheza* Enghoff, Golovatch & Nguyen, 2004 or *Kronopolites* Attems, 1914. In addition, an identification key to species of *Oxidus* is provided.

**Keywords.** Millipedes, Diplopoda, *Oxidus*, molecular phylogeny, revision.
Introduction

The genus *Oxidus* was proposed for the species *Fontaria gracilis* C.L. Koch, 1847 by Cook (1911). However, Attems (1914) also proposed a subgenus, *Kalorthomorpha*, of the genus *Orthomorpha* Bollman, 1893 for the same species. Later, *Kalorthomorpha* was elevated to full generic rank and considered to be a junior synonym of *Oxidus* (Jeekel 1968).


Chamberlin & Wang (1953) referred all *circofera*, *gracilis*, *pekuensis*, *obtusus* and *nordenskiöldi* to the genus *Oxidus*. However, Takakuwa (1954) listed only four species of *Oxidus*, *O. gracilis*, *O. avia*, *O. riukiaria* and *O. obtusus*, for the Japanese fauna. Wang (1957) added another species, *Oxidus* (*Varyomorpha*) *hsientienensis* Wang, 1957 (= *Nedyopus hsientienensis* according to Chen *et al.* (2006)) from Taiwan. Miyosi (1959) considered only five species *Oxidus gracilis*, *O. circofera*, *O. nordenskioeldi*, *O. cristatus* and *O. obtusus* from Japan.

Jeekel (1963a, 1968) accepted the validity of the genus *Oxidus*, and listed only five species and subspecies, *O. avia*, *O. gracilis*, *O. gracilis* ssp. *gigas* (Attems, 1953), *O. obtusus* and *O. riukiaria*. *O. gracilis* *gigas* was poorly described as *Kalorthomorpha gracilis gigas* from North Vietnam (Attems 1953), and recently raised to full species rank by Enghoff *et al.* (2004).

*O. gracilis* (C.L. Koch, 1847) is a cosmopolitan species; *O. obtusus* (Takakuwa, 1942) was known from South Korea, both *O. avia* (Verhoeff, 1937) and *O. riukiaria* (Verhoeff, 1940) were reported from South Japan, and lastly *O. gigas* (Attems, 1953) was recoded from North Vietnam. Except for *O. gracilis*, all the remaining species have had no further records since their establishment. This paper seeks to answer three questions based on morphological and molecular analyses. Morphological data is used to classify species and molecular data is used to examine their phylogeny.

1) How many species are actually in the genus *Oxidus*?
2) What are the relationships among species of *Oxidus*?
3) Does the genus *Oxidus* form a monophyletic group with closely related groups, e.g., *Tylopus* and *Sellamuchea*?
Material and methods

Taxon sampling, identification and DNA extraction

Examined material was collected by hand in northern Vietnam and southern Japan, or was borrowed from the collections in Kyungpook National University (KNU) (Korea) and the Hungarian Museum of Natural History (HNHM). All were examined under an Olympus SZX10 microscope with a drawing tube.

Total genomic DNA was extracted from leg tissue using the DNAeasy Blood & Tissue Kit (Qiagen TM).

DNA amplification and sequencing

Fragments of two mitochondrial genes, cytochrome c oxidase subunit I (COI) and 16S rRNA, were amplified using polymerase chain reaction (PCR). Universal primers LCO-1490 and HCO-2918 (Folmer et al. 1994) or COI-1F20 (5’-ACT CTA CTA ATC ATA AGG AT-3’) and COI-1R19 (5’-TAA ACC TCC GGG TGA CCAA-3’) were used to amplify a 680 bp fragment of the COI gene. Primer sets 16Sa (5’- CGC CTG TTT AHC AAA AAC AT-3’) -16Sb (5’-CCG GTY TGA ACT CAR ATC CA-3’) or 16S-1F19 (5’- CCG GTT TGA ACT CAG ATCA-3’) and 16S-1R20 (5’-TGA CTG TTT AGC AAA GAC AT-3’) were used to amplify a 550 bp fragment of the 16S rRNA gene. PCR conditions for amplification of the 16S rRNA gene were: an initial denaturation at 95°C for 2 min followed by 36 cycles of 95°C for 20 sec, 45°C for 40 sec and 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR conditions for the amplification of COI were: initial denaturation at 94°C for 5 min followed by 38 cycles of 94°C for 45 seconds, 42°C for 45 seconds, 72°C for 90 seconds, and a final extension at 72°C for 5 min. After thermal cycling, 2 µl PCR products were screened for potentially successful amplification of a fragment of 16S or COI through electrophoresis in 1% agarose-TBE 1X. The electrophoresis was performed at conditions of 100 mA and 120 V for 1 hour.

About 20 µl of successfully amplified PCR products were purified using ExosapIT or the QIAquick PCR Purification Kit (Qiagen Inc.). Purified PCR products were sequenced at Solgen, Inc (Korea) in an Applied Biosystems automatic sequencer (ABI3130 XL) using the same primer sets used for initial PCR.

Alignment and phylogenetic analysis

Each successful sequence was manually checked using BioEdit ver. 7.1 (Hall 1999) and confirmed by BLAST searches (Altschul et al. 1990). All confirmed sequences were aligned with MUSCLE (Edgar 2004). Ambiguous nucleotide sites and gaps were removed using MEGA ver. 6.0 (Tamura et al. 2013). The reliability of the alignment was estimated using distance estimation and model of p-distance. Nucleotide frequencies were statistically calculated using MEGA 6.0. These COI sequences were translated into amino acids for confirmation using transversion code in MEGA 6.0.

Model test was implemented in MEGA 6.0 to find the most appropriate maximum likelihood substitution model for COI and 16S. Models with the lowest Bayesian Information Criterion (BIC) scores were considered for describing the best substitution pattern for each gene. Codon positions included were: 1st + 2nd + 3rd + Noncoding. The selected model for COI was the Tamura-Nei model + G + I (Tamura & Nei 1993). For the combination of COI and 16S rRNA, the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) was used. Phylogenetic trees were constructed using both maximum likelihood (ML) and Bayesian inference (BI) models. Maximum likelihood bootstrap analysis was conducted using MEGA 6.0 with 1000 replicates. A Bayesian inference (BI) tree was created using MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist 2001) with 10 million generations, heating parameter of 0.06, and sampling every 1000 generations. All nucleotide sequences were deposited at GenBank. Collection localities, specimen –
The COI dataset consisted of 525 bp sequences from 18 taxa. The 16S rRNA dataset included 466 bp sequences from 13 taxa. The combination of 16S and COI genes contained 991 bp sequences from 12 taxa (Table 2). The incongruence length difference (ILD) test showed congruence of genes COI and 16S. Therefore, the analyses were performed for gene COI and the combination of 16S and COI. Species of the genus *Tonkinosoma* Jeekel, 1953 (tribe Tonkinosomatini Jeekel, 1968) were employed as an outgroup.

Abbreviations used in text and figures

The terminology for the genus *Oxidus* follows that used by Likhitrakarn et al. (2010) for the genus *Tylopus*:

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Table 1. Details of specimens analysed for DNA, including GenBank numbers.

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Locality</th>
<th>Voucher</th>
<th>16S rRNA</th>
<th>COI</th>
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<tr>
<td>1</td>
<td><em>Oxidus gigas</em> (Attems, 1953)</td>
<td>Sapa, Lao Cai, Vietnam</td>
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<td>Tam Dao, Vinh Phuc, Vietnam</td>
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<td><em>Tylopus crassipes</em> Golovatch, 1984</td>
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<td>IEBR-Myr 92</td>
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<td><em>Kronopolites montanus</em> Golovatch, 2009</td>
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<td>IEBR-Myr 175</td>
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<td>Cat Ba, Hai Phong, Vietnam</td>
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NGUYEN A.D. et al., Revision of the genus Oxidus Cook, 1911

ca = cannula
co = coxa
pf = prefemorite
fe = femorite
l = postfemoral lamina
z = postfemoral spine
h = postfemoral process
sl = solenomere
sph = solenophore

Institutional abbreviations
All materials and voucher specimens are kept in:
IEBR = Institute of Ecology and Biological Resources, Hanoi, Vietnam
IPE = Institute of Phylogenomics and Evolution, Daegu, Korea
KNU = Kyungpook National University, Daegu, Korea
HNHM = Hungarian Natural History Museum, Budapest, Hungary

Results

Order Polydesmida Leach, 1813
Family Paradoxosomatidae Daday, 1889
Tribe Sulciferini Jeekel, 1968

Genus Oxidus Cook, 1911

Oxidus Cook, 1911: 628.
Kalorthomorpha Attems, 1914: 195 (proposed as subgenus of Orthomorpha Bollman, 1893; junior objective synonym of Oxidus, see Jeekel 1968: 71).

Oxidus – Brölemann 1916: 537.

Type species
Fontaria gracilis C.L. Koch, 1847, by original designation.

Diagnosis
The genus can be distinguished from other genera in the tribe Sulciferini by the combination of characters: paraterga being well-developed; metaterga with one or two rows of setae; legs without tarsal brushes; 5th sternum without modifications.

Gonopod relatively complicated; femorite weakly twisted, slightly or strongly enlarged distally, strongly grooved mesally, well demarcated laterally from postfemoral lamina l by a distinct sulcus; solenophore with two basal processes: long, highly elevated process h and short, spiniform/tuberculiform, sub-transverse process z; solenophore twisted or strongly spiral, with mesal well-developed lobule, but without lateral lobules; solenomere flagelliform, completely sheathed by solenophore.

Remarks
This genus is distributed in the northern part of the Oriental and the eastern part of the Palearctic regions. It is unlikely that the genus is of tropical origin because it is absent from the tropical regions in the Oriental (Jeekel 1963a). Its center of origin is more likely to be Japan (Jeekel 1968).
The genus *Oxidus* was placed in Sulciferini in view of the characters of the spiral solenophore completely sheathing the solenomere, the presence of a postfemoral demarcation and of postfemoral processes (Jeekel 1968). Golovatch & Enghoff (1993) recommended that the genus should be placed relatively close to the genus *Tylopus* Jeekel, 1968, rather than to other sulciferinine genera. The phylogeny of the genus *Oxidus* is discussed below.

*Oxidus gracilis* (C.L. Koch, 1847)
Figs 1–2

*Fontaria gracilis* C.L. Koch, 1847: 142.

*Fontaria gracilis* – Koch C.L. 1863: 51, pl. 85, fig. 173.
*Orthomorpha* (*Kalorthomorpha*) *gracilis* – Attems 1914: 196.

![Image](image.png)

**Fig. 1.** *Oxidus gracilis* (C.L. Koch, 1847) from Okinawa Island, Japan. **A.** Entire body, length ca 23 mm. **B–C.** Segments 8–9–10. **B.** ♂. **C.** ♀. Scale bars = 1 mm. (photos by Z. Korsós)
Material examined

JAPAN: 1 ♂, Central Ryukyu, Okinawa-jima Island, Onna-son, Onna, Camp Hansen training area at Gate 28, 20 m a.s.l., 26°29′44″ N, 127°51′36″ E, 31 Aug. 2010, leg. Z. Korsós (IEBR-501); 3 ♂♂, 3 ♀♀, Central Ryukyu, Okinawa-jima, Okuni rindo forest trail, 26°43′55″ N, 128°12′30″ E, 25 Sep. 2012, leg. Z. Korsós (IEBR-H471); 3 ♂♂, 3 ♀♀, Central Ryukyu, Okinawa-jima, Nago city, Makiya, Makiya-notaki waterfall, 26°37′44″ N, 128°02′42″ E, 100 m, 2 Sep. 2012, leg. Z. Korsós (IEBR-H466).


TAIWAN: 1 ♂, Miaoli County, Taian Township, Kuanwu, SE ridge of Mt. Yemagan, 24°31′13″ N, 121°07′11″ E, 1925 m, secondary mixed forest, 20 Oct. 2009, leg. L. Dányi and E. Lazányi (HNHM-T09-51); 1 ♂, 1 ♀, Taichung County, Heping Township, Dasyueshan Forest Recreation Areas, Shuehshan Rd., 7 May 2003, leg. Shining Wu (IEBR-Myr 550); 3 ♂♂, 1 ♀, Miaoli County, Taian Township, Dalu Forest Rf., West Feeder, Guanwu lodge, 12 Aug. 2002, leg. Chen Chao Chun (IEBR-Myr 551).

Fig. 2. Left gonopod of Oxidus gracilis (C.L. Koch, 1847), sample IEBR-USA. A. Lateral view. B. Mesal view. Scale bars = 100 μm.
Diagnosis
This species differs from its congeners in the gonopod femorite being strongly expanded distally, not cylindrically slender; postfemoral lamina rectangular; spine pointed tuberculiform; process lamellar, slightly suberect, but bent upwards from midpart, serrated at distolateral part; both bases of process and spine clearly separated; mesal lobule of solenophore very well-developed, lamella-shaped, distinctly separated from tip of solenophore; tips of both mesal lobule and solenophore circularly emarginated.

DNA
For *Oxidus gracilis*, DNA data was collected for three genes (mitochondrial COI gene, nuclear 18S and 28S rRNA genes), but there is no data from the mitochondrial 16S rRNA. In this study, two fragments of COI and 16S were sequenced and deposited in GenBank (see Table 1).

Distribution

*Oxidus gigas* (Attems, 1953)
Figs 3–4

*Kalorthomorpha gracilis gigas* Attems, 1953: 165, fig. 44.

*Oxidus gracilis* – Golovatch 1984: 54.  

Diagnosis
This species is particularly similar to *Oxidus gracilis* (C.L. Koch, 1847) in body appearance and gonopod conformation, but differs in larger size and in details of gonopod structure: process narrow and long, pointed at the end and not serrated at distolateral margin; bases of process and spine less distinctly separated.

Material examined

Redescription
*Size.* Body length 31–34.5 mm (♂), 29.9–30.2 mm (♀); width of midbody pro- and metazonae 1.9–2.2 mm (♂), 2.2 mm (♀) and 2.8–3.2 mm (♂), 3.1–3.2 mm (♀), respectively.
COLORATION. Generally castaneous brown, but anterior half of metaterga and posterior margin of prozonae darkish brown. Posterior half of metaterga, anterior margin of prozonae and pleura castaneous brown; paraterga, sterna and legs brownish yellow.

HEAD. Slightly narrower than collum; labrum sparsely setose; epicranial suture distinct, dividing frons into two equal parts. Antennae long and slender, not claviform, reaching segment 3 if stretched posteriorly. Antennomere 1<7<2=3=4=5=6 in length.

COLLUM. Subequal to, or slightly narrower than collum, trapeziform; surface shining and smooth, without rugosity; setae broken, but traces of two rows: 3+3 close to anterior margin and 1+1 in middle. Paraterga large, ear-shaped with broadly rounded laterocaudal corners; lateral side with a setiferous incision.

In width, segment 4≤3<2<5–17, thereafter gradually tapering towards telson. Prozonae and metaterga shining, smooth, without rugosity. Metaterga with two rows of setae: 2+2 close to anterior margin and

![Image of Oxidus gigas](image-url)

**Fig. 3.** *Oxidus gigas* (Attems, 1953) from Duc Xuan Commune, Ha Giang Prov., Vietnam. A. Entire body, length ca 34 mm. B–C. Segments 8–9–10. B. ♂. C. ♀. Scale bars = 1 mm. (photo by Anh Nguyen)
2+2 close to posterior margin. Transverse sulcus starting on metatergum 4, well developed, reaching base of paraterga on metaterga 5–19, beaded at bottom on some caudal segments. Stricture between pro- and metazonae clearly distinct, broad and striolate at bottom. Pleura shining, smooth; pleurosternal carinae completely absent or poorly developed only on pre-gonopodal segments.

**Paraterga.** Well developed, lying subequal to or lower than metatergal surface. Calluses small, but obvious; lateral side with 2 setiferous incisions at ⅓ and ⅔ of its length. Anterior corner broadly rounded, but posterior corner acute, produced into a pointed projection on segments 15–17. Ozopores located behind second lateral incision of paraterga 5, 7, 9, 10, 12, 13, 15–19.

**Epiproct.** Long, but broadly truncated, dorsoventrally flattened, with two minute apical tubercles. Tip with four spinnerets. Hypoproct trapeziform, with two well separated, distolateral, setiferous knobs.

**Sterna.** Modestly setose, without modifications, but with a minute cone caudally near each coxa.

**Leg.** Long and slender, about 1.6–1.7 (♂), 1.4–1.5 (♀) times as long as midbody height. Tarsal brushes absent. Prefemora not swollen. Femora without modifications.

**Gonopod.** Relatively complicated. Coxite cylindrical, long, subequal to femorite in length; distoventral part sparsely setose. Prefemorite densely setose, separated laterally from femorite by a distinct oblique sulcus. Femorite weakly twisted, grooved mesally, more or less slightly enlarged distally, without modifications and demarcated from postfemoral region by a lateral sulcus. Postfemoral region shorter than femorite, with a lateral sub-pentagonal lamina l; spine z pointed; process h long and pointed, not serrated laterally; bases of process h and spine z not separated. Solenophore strongly spiral; distomesal lobule well developed and distinctly separated from solenophore tip. Solenomere flagelliform, completely sheathed by solenophore.

![Fig. 4.](image-url) Right gonopod of Oxidus gigas (Attems, 1953), from sample IEBR-Myr 113. A. Lateral view. B. Ventral view. C. Mesal view. The picture has been flipped horizontally. Scale bars = 1 um.
DNA
COI and 16S barcode data (partial) are deposited in GenBank (Table 1).

Habitats
All material was found under leaf-litter, logs and decaying wood.

Distribution
The species has only been recorded from northern Vietnam (Lao Cai, Ha Giang and Vinh Phuc Provinces).

Remarks
Attems (1953) proposed a new subspecies Kalorthomorpha gracilis gigas with a short note. Enghoff et al. (2004) raised this subspecies to full rank as Oxidus gracilis, and also showed minor differences in size and gonopod process h. The species is fairly similar to O. gracilis, but the molecular data has provided strong evidence to separate the species.

Golovatch (1984) misidentified O. gracilis in Vietnam (sample IEBR-H133), which is currently corrected as O. gigas in this paper.

**Oxidus riukiaria** (Verhoeff, 1940)
Figs 5–7

Orthomorpha riukiaria Verhoeff, 1940: 139, figs 5–6.

Orthomorpha riukiaria – Takakuwa 1954: 39, fig. 34.

Material examined

JAPAN: 4 ♂♂, 4 ♀♀, Central Ryukyus, Okinawa-jima, Ogimi village, Nerome, above road no.58, 26°41’37″ N, 128°06’43″ E, 37 m a.s.l., 25 Sep. 2012, leg. Z. Korsós (IEBR-H470); 2 ♂♂, 2 ♀♀, Okinawa-jima, Yanbaru, Ogimi village, Nunha, Okuni rindo, 26°41’10.1″ N, 128°09’02.7″ E, 15 Sep. 2012, leg. Nakamura Y. (IEBR-H500); 2 ♂♂, Central Ryukyus, Okinawa Island, Yanbaru, Kunigami village, Cape Hedo, Ginama, broad-leaved evergreen forest, 176 m, 26°49’52″ N, 128°16’20″ E, 5 Apr. 2011, leg. Z. Korsós (HNHM-355); 1 ♂, Northern Ryukyus, Tokara group, Kuchino-shima Island, around Kuroshio-so guesthouse, 29°59’24″ N, 129°55’21″ E, 63 m, 14 Oct. 2012, leg. Z. Korsós (HNHM-477).

**Other records** (newly found ones underlined)

JAPAN: Northern Ryukyus, Tokara Group: Kuchino-shima Island; Central Ryukyus: Okinawa-jima, Kume-jima, Iheya-jima, Aguni-jima, Zamami-jima, Aka-jima, Geruma-jima (observations by Z. Korsós).

**Diagnosis**

Oxidus riukiaria can be distinguished from all other species of Oxidus by its smaller size (19.3–20.6 mm in length, width of pro- and metazonae 1.5–1.8 mm and 2.1–2.2 mm); gonopod coxae more or less stouter and longer than femorite; femorite narrow at base, and strongly expanded towards distal end; gonopod process h hook-like, larger and getting narrower towards pointed tip, spine z small, pointed spiniform; postfemoral lamina l triangularly rounded; mesal lobule not seperated from solenophore tip, which is distinctly emarginated.
DNA
COI and 16S barcode data (partial) are deposited in GenBank (Table 1).

Remarks
This species was originally described by Verhoeff (1940) in the genus *Orthomorpha*, but was assigned to *Oxidus* by Jeekel (1963a). The taxonomic position of the species is also well supported by molecular data.

Distribution
South Japan (Ryukyu Islands).

*Oxidus avia* (Verhoeff, 1937)
Fig. 8A

*Orthomorpha avia* Verhoeff, 1937: 33, fig. 1.
Fig. 6. Left gonopod of *Oxidus riukiaria* (Verhoeff, 1940). A. Lateral view. B. Postfemoral region, lateral view. Redrawn from Verhoeff 1940. No scale bars.

Fig. 7. Left gonopod of *Oxidus riukiaria* (Verhoeff, 1940), from sample IEBR-H470. A. Lateroventral view. B. Ventral view. C. Postfemoral region, ventral view. Note: z = spine z, but broken.

**Diagnosis**

This species can be separated from all other species of *Oxidus* by its much darker, almost uniformly dark brown body, slender base of gonopod solenophore, more clearly and obtusely emarginated posterior edge of telson; smaller postfemoral lamina l; stouter spine z; process h more erect, slender and pointed; the base of solenophore less protruded, more strongly rounded; mesal lobule distinctly separated from rounded solenophore tip.

**Remarks**

The species was originally described by Verhoeff (1937) under the genus *Orthomorpha*, then was assigned to the genus *Oxidus* by Takakuwa (1954).

**Distribution**

South Japan (Ryukyu Islands): Ishigaki-jima, Yonaguni-jima; Taiwan.

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**Fig. 8.** A. Left gonopod of *Oxidus avia* (Verhoeff, 1937), lateral view. B. Right gonopod of *Oxidus obtusus* (Takakuwa, 1942), mesal view. Redrawn from Verhoeff 1937 and Takakuwa 1942a, respectively. No scale bars.
Oxidus obtusus (Takakuwa, 1942)

Fig. 8B

Orthomorpha obtusa Takakuwa, 1942a: 363, 367, fig. 7.


Diagnosis

This species can be distinguished by its gonopod femorite being short, very broad gently distad; process \( h \) bent upward at tip, like a lamellar sickle; spine \( z \) small, obtuse; solenophore large, broad and simple, especially wide at its base; mesal lobule not separated from rounded solenophore tip (Takakuwa 1942a).

Remarks

This species was described from South Korea (Takakuwa 1942a). Later, Chamberlin & Wang (1953) reported two ♀♀ in the collection of the American Natural History Museum (New York) from Japan. It is possible that the locality was mislabelled because they stated that those specimens were collected and deposited by Dr. Takakuwa Y. Thus, it is believed that these two ♀♀ were collected from the same locality as the species holotype (South Korea).

Lim (2001) synonymized *O. obtusus* with *O. gracilis*. He argued that the two species differ only in the number of postfemoral branches, three in *O. obtusus* and four in *O. gracilis*; in his opinion, such a difference is minor and cannot be used to separate two species. With the two species *O. gracilis* and *O. gigas*, those characters are important for species delimitation. It is recommended, therefore, that *Oxidus obtusus* should be considered a valid species. Examination of further fresh material is needed to confirm that recommendation.

Distribution

South Korea.

Key to species of the genus Oxidus Cook, 1911

1. Gonopod femorite stout, more or less cylindrical. Spine \( z \) small and short, blunt spiniform .......................... *O. obtusus* (Takakuwa, 1942)
   – Gonopod femorite strongly expanded distad, not cylindrical. Spine \( z \) long, pointed tuberculiform or spiniform .......................... 2

2. Postfemoral lamina \( l \) small, spiniform. Spine \( z \) stout, tip more or less rounded, tuberculiform. Base of solenophore slender; mesal lobule clearly separated from rounded tip of solenophore .......................................................... *O. avia* (Verhoeff, 1937)
   – Postfemoral lamina \( l \) large, not spiniform. Spine \( z \) pointed spiniform. Base of solenophore not slender; tip of solenophore emarginated .................................................... 3

3. Postfemoral lamina \( l \) triangular. Mesal lobule not separated from solenophore tip ..............
   ................................................................. *O. riukiaria* (Verhoeff, 1940)
   – Postfemoral lamina \( l \) not triangular. Mesal lobule clearly separated from solenophore tip ........ 4

4. Postfemoral lamina \( l \) sub-pentagonal. Process \( h \) and spine \( z \) not separated clearly at base of postfemoral region. Process \( h \) pointed ........................................ *O. gigas* (Attems, 1953)
   – Postfemoral region \( l \) rectangular. Process \( h \) and spine \( z \) clearly separated at base of postfemoral region. Process \( h \) serrated along upper edge .......................... *O. gracilis* (C.L. Koch, 1847)
Molecular phylogeny of the genus *Oxidus* Cook, 1911

DNA variations and distances

The aligned dataset for COI consisted of 525 bp sequences. The nucleotide frequencies for A, T, G, and C were 20.6%, 42.4%, 23.0%, and 13.9%, respectively. The GC content was 37%. The COI dataset contained 157 (29.9%) parsimony informative and 177 (33.7%) variable sites (see Table 2). The uncorrected p-distance between taxa ranged from 0.000 to 0.192. The overall p-distance was 0.146.

The aligned dataset for the 16S rRNA gene contained 466 bp sequences. The nucleotide frequencies of A, T, G, and C were 31.9%, 35.7%, 24.0%, and 8.4%, respectively. The GC content was 32.4%. The 16S rRNA dataset contained 161 (14.7%) parsimony informative and 199 (34.7%) variable sites (see Table 2). The uncorrected p-distance between the taxa ranged from 0.000 to 0.279. The overall p-distance was 0.169.

The aligned dataset for the combination of the 16S rRNA and COI genes contained 991 bp sequences. The nucleotide frequencies of A, T, G, and C were 26.0%, 39.1%, 23.3%, and 11.6%, respectively. The GC content was 34.9%. This combined dataset contained 297 (30.0%) parsimony informative and 367 (37.0%) variable sites (see Table 2). The uncorrected p-distance between the taxa ranged from 0.000 to 0.229. The overall p-distance was 0.157 (Table 3).

Within the genus *Oxidus*, the three species compared had significant genetic distances: 0.106-0.113 between *O. gigas* and *O. gracilis*; 0.119-0.128 between *O. gigas* and *O. riukiaria*; and 0.103-0.106 between *O. gracilis* and *O. riukiaria*. The genetic distance between species of *Oxidus* and other genera ranged from 0.162 (between *O. riukiaria* and *Tylopus* roseiparaterga) to 0.229 (between *O. gigas* and *Kronopolites* sp.).

Phylogenetic analyses

Phylogenetic trees were reconstructed for the COI and 16S-COI combination using ML and BI. For ML-analysis, we considered clades with bootstrap values below 65%, between 65 and 89%, or more than 89% to be weakly supported, moderately supported, or strongly supported, respectively (Pimvichai et al. 2014). For BI-analysis, clades with a BI posterior probability less than 0.7 bpp, between 0.7 and 0.95 bpp, or more than 0.95 bpp are considered to be weakly supported, moderately supported, or strongly supported, respectively.

Based on a Bayesian Inference and Maximum Likelihood analysis of a 525 bp fragment of COI from 18 taxa, a phylogenetic tree was reconstructed (Fig. 9). The genus *Oxidus* formed a monophyletic clade. It was separated from its sister group (genus *Tylopus*) with an ML bootstrap value of < 65% and a BI posterior probability of 0.76 bpp. *Sellanucheza* is considered as the sister group of *Tylopus* plus *Oxidus*. The separation of *Sellanucheza* and (*Tylopus* plus *Oxidus*) is supported with an ML bootstrap value of < 65% and a BI posterior probability of 0.98 bpp. Within *Oxidus*, the three species formed three clearly distinguished clades. However, *O. gigas* was more closely related to *O. gracilis*, with an ML bootstrap value of 74% and a BI posterior probability of 0.86 bpp. *O. riukiaria* was separated from the group of

<table>
<thead>
<tr>
<th>Genes</th>
<th>Average base frequencies</th>
<th>Length</th>
<th>Variable sites</th>
<th>Parsimony informative sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>T</td>
<td>G</td>
<td>C</td>
</tr>
<tr>
<td>COI</td>
<td>20.6</td>
<td>42.4</td>
<td>23.0</td>
<td>13.9</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>31.9</td>
<td>35.7</td>
<td>24.0</td>
<td>8.4</td>
</tr>
<tr>
<td>16S-COI combination</td>
<td>26.0</td>
<td>39.1</td>
<td>23.3</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Table 2. Average base frequencies of two partial genes COI, 16S rRNA and the 16S-COI combination.
NGUYEN A.D. et al., Revision of the genus *Oxidus* Cook, 1911

**Table 3.** Uncorrected distance of the combination of COI and 16S rRNA genes calculated by MEGA 6.0. The genetic distances among species of *Oxidus* are highlighted in bold.

<table>
<thead>
<tr>
<th>Species / p-distance</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Tonkinosoma jeekeli (545)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>2 Kronopolites montanus (175)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.196</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Kronopolites sp. (IPE4)</td>
<td></td>
<td>0.196</td>
<td></td>
<td></td>
<td>0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Tylopus crassipes (92)</td>
<td>0.201</td>
<td>0.225</td>
<td>0.220</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>5 Tylopus roseiparaterga (185A)</td>
<td>0.205</td>
<td>0.207</td>
<td>0.203</td>
<td>0.119</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>6 Oxidus gigas (516)</td>
<td>0.201</td>
<td>0.225</td>
<td>0.229</td>
<td>0.163</td>
<td>0.186</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7 Oxidus gigas (133)</td>
<td>0.203</td>
<td>0.224</td>
<td>0.223</td>
<td>0.172</td>
<td>0.187</td>
<td>0.040</td>
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</tr>
<tr>
<td>8 Oxidus gracilis (466)</td>
<td>0.188</td>
<td>0.205</td>
<td>0.201</td>
<td>0.166</td>
<td>0.172</td>
<td>0.113</td>
<td>0.108</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>9 Oxidus gracilis (471)</td>
<td>0.190</td>
<td>0.205</td>
<td>0.201</td>
<td>0.169</td>
<td>0.172</td>
<td>0.113</td>
<td>0.106</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Oxidus gracilis (USA)</td>
<td>0.188</td>
<td>0.205</td>
<td>0.201</td>
<td>0.166</td>
<td>0.172</td>
<td>0.113</td>
<td>0.108</td>
<td>0.000</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Oxidus riukiaria (500)</td>
<td>0.186</td>
<td>0.208</td>
<td>0.208</td>
<td>0.166</td>
<td>0.170</td>
<td>0.128</td>
<td>0.125</td>
<td>0.106</td>
<td>0.104</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td>12 Oxidus riukiaria (500J)</td>
<td>0.186</td>
<td>0.204</td>
<td>0.206</td>
<td>0.169</td>
<td>0.162</td>
<td>0.128</td>
<td>0.119</td>
<td>0.104</td>
<td>0.103</td>
<td>0.104</td>
<td>0.037</td>
</tr>
</tbody>
</table>

*O. gigas* and *O. gracilis* with a well supported ML bootstrap value of 98% and a BI posterior probability of 1.00 bpp.

Based on BI and ML analysis of the 991bp combination of the genes 16S rRNA and COI, a phylogenetic tree of 12 taxa was constructed (Fig. 10). As in the COI tree, the genus *Oxidus* formed a monophylic clade clearly separated from species of *Tylopus*, with a well supported ML bootstrap value of 100% and a BI posterior probability of 1.00 bpp. The three *Oxidus* species were also separated from each other. *O. riukiaria* formed a sister group with *O. gigas* plus *O. gracilis*, with an ML bootstrap value of 100% and a BI posterior probability of 1.00 bpp. The species *O. gigas* was separated from *O. gracilis* with a moderately supported ML bootstrap value of 83% and a BI posterior probability of 0.91 bpp.

**Discussion**

Both phylogenetic trees showed the monophyly of each *Oxidus* species (Figs 9–10). In other words, all three species are distinctly separated from each other (genetic distances: 0.106–0.113 between *O. gigas* and *O. gracilis*; 0.119–0.128 between *O. gigas* and *O. riukiaria*; and 0.103–0.106 between *O. gracilis* and *O. riukiaria*). The species *O. gigas* was originally proposed as a subspecies of *O. gracilis* (Attems, 1953). After being raised to full species rank, it is still closer to *O. gracilis* than to other species of *Oxidus*. This relationship is well supported by a monophyletic clade of the two species, *O. gracilis* and *O. gigas*. The other species, *O. riukiaria*, is considered as the sister species of *O. gracilis* plus *O. gigas*. 

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The genus *Oxidus* is placed in the Sulciferini with the typical characters of a spiral solenophore completely sheathing the solenomere, presence of postfemoral demarcation, and postfemoral processes (Jeekel 1968). Until now, no relationship analysis among sulciferine genera has been reported, except that Golovatch & Enghoff (1993) have reported a close relationship among species of *Tylopus* and *Oxidus gracilis*. These authors recommended that *Oxidus gracilis* or the genus *Oxidus* should be considered as the sister of genus *Tylopus* because both genera have similar gonopod characters, such as the presence of lamina l, process h, and spine z. Molecular analysis based on the COI gene and the combination of 16S and COI genes provides good supports for the recommendation of Golovatch & Enghoff (1993). In both phylogenetic trees, *Oxidus* and *Tylopus* formed a group with well supported bootstrap value and BI posterior probability (Figs 9–10).

The genus *Sellanucheza* Enghoff, Golovatch & Nguyen, 2004 was originally assigned to the tribe Tonkinosomatini (Enghoff et al. 2004). However, Golovatch (2013) suggested that it was better to place this genus in the tribe Sulciferini. His suggestion is well supported by the molecular data obtained from this study. The genus *Sellanucheza* is relatively close to the group of *Tylopus* + *Oxidus*, with a very high BI value (0.98 bpp) in the phylogenetic tree inferred from the 525 bp fragment of the COI gene (Fig. 10).

**Conclusion**

The genus *Oxidus* Cook, 1911 consists of five species, *O. avia*, *O. gigas*, *O. gracilis*, *O. riukiaria*, and a doubtful species, *O. obtusus*. While *O. gracilis* is a cosmopolitan species, widely found in temperate and sub-tropical regions over the world, the other species have limited distributions in particular regions: *O. gigas* in northern Vietnam, and *O. riukiaria* and *O. avia* in the Ryukyu Islands (Japan).

![Fig. 9. Phylogenetic tree of the genus Oxidus and some closely related groups based on Maximum Likelihood and Bayesian Inference Analysis of a 525 bp fragment of the COI gene (# = a value less than 65%).](image-url)
Oxidus is a monophyletic genus, and most closely related to the genus Tylopus based on the molecular analysis. All three species, O. gigas, O. gracilis and O. riukiaria, are distinctly separated and O. gigas is closer to O. gracilis than to O. riukiaria.

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References


Fig. 10. Phylogenetic tree of the genus Oxidus and some closely related groups based on Maximum Likelihood and Bayesian Inference Analysis of a 991 bp fragment of the combination of 16S rRNA and COI genes.


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