Xenia konohana sp. nov. (Cnidaria, Octocorallia, Alcyonacea), a new soft coral species in the family Xeniidae from Miyazaki, Japan

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Abstract

A new soft coral species, Xenia konohana sp. nov. (Alcyonacea, Xeniidae), is described from Miyazaki in the warm-temperate region of Japan. This new species has conspicuous and unique spindle sclerites in addition to the simple ellipsoid platelet-shaped sclerites typically found in the genus Xenia. These unique spindles are a specific key morphological characteristic for this new species and for differentiating this species among congeneric species.

Keywords

Alcyonacea, Cnidaria, Miyazaki, new species, Xenia, Xeniidae

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**Introduction**

Species of the family Xeniidae are known as pioneers in tropical coral reefs (Benayahu and Loya 1987), playing an important role for ecological succession in coral reefs. Therefore, knowing how many species of Xeniidae exist, and the range of species diversity will be useful for understanding the coral reef ecosystem.

For species or genus identification of alcyonacean soft corals including xeniids, the shape and arrangement of sclerites are used as key characteristics. Xeniids typically produce minute platelets or corpuscle-like sclerites without tubercular differences among species and genera under light microscopy (Fabricius and Alderslade 2001). The microstructure of sclerites has been shown to be an important character at the genus level of the family Xeniidae. Recently, the type specimens of 21 species in the genus *Xenia* were rechecked and re-described using sclerite microstructure (Halász et al. 2019). Thus, observation of sclerite microstructure is taxonomically useful for species delimitation, at least in some species of *Xenia*.

The genus *Xenia* presently includes 49 valid species (Cordeiro et al. 2021). This genus is characterized by platelet-shaped sclerites with surface microstructure composed of calcite dendritic and sinuous rods (Alderslade 2001; Halász et al. 2019). Koido et al. (2019) reported an undescribed species belonging to *Xenia* (reported as *Xenia* sp. 1) from Oshima Island, Miyazaki, in the warm-temperate region (non-coral reef region) of Japan. This previous work emphasized the high species diversity of Xeniidae in Miyazaki, Japan. This study provides a description of this previously undescribed species (*Xenia* sp. 1) as *Xenia konohana* sp. nov., a new species in the genus.

**Materials and methods**

All specimens were collected around Oshima Island (31°31.35’N, 131°24.27’E) (Fig. 1), Miyazaki, Japan, by SCUBA diving and snorkeling. A small piece of tissue (5–10 mm) from each specimen was used for molecular analyses and the remainder was preserved in 99% ethanol for morphological analyses as reported by Koido et al. (2019).

Specimens were previously deposited in Miyazaki University, Fisheries Sciences (MUFS) but were subsequently transferred and deposited at the Kuroshio Biological Research Foundation, Kochi, Japan (KBF) in the octocoral collection (OA). Morphological characteristics examined under a stereomicroscope included colony height, length and width of the stalk, presence of branches, length and width of polyps, length and width of tentacles, length and width of pinnules, number of rows of pinnules, and number of pinnules in the aboral row. Sclerites from polyps, and ones from the surface and interior of both stalk and branches of each specimen were examined. Sclerite shape, size, and microstructure were examined with light microscopy and scanning electron microscope (SEM) (HITACHI S-4800 and JEOL JSM-6500F).
DNA extraction, amplification, and sequencing

Tissue samples were kept in CHAOS solution for at least a week to dissolve proteins at room temperature as reported by Koido et al. (2019). Total DNA was extracted from CHAOS solutions by conventional phenol/chloroform extraction. The phylogenetic position of *X. konohana* sp. nov. was inferred using three mitochondrial markers (*ND2*, *mtMutS*, *COI*) (16S647F: 5’-ACA CAG CTC GGT TTC TAT CTA CCA-3’; ND21418R: 5’ -ACA TCG GGA GCC CAC ATA-3’, ND42625F: 5’-TAC GTG GYA CAATGG CTG-3’, Mut-3458R: 5’-TSG AGC AAA AGC CACTCC-3’, COII8068F: 5’-CCA TAA CAG GAC TAG CAG CAT C-3’, HC02198: 5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’) and a nuclear marker (*28S*) (28S-Far: 5’-CAC GAG ACC GAT AGC GAA CAA GTA-3’, 28S-Rar: 5’-TCA TTT CGA CCC TAA GAC CTC-3’). PCR reactions for all four markers used 1 μL of DNA solution, 1.6 μL of 2.5 mM dNTP Mixture, 2 μL of 10X Ex Taq buffer, 2 μL of each primer (10 mM), 0.08 μL Ex Taq (TaKaRa), and 11.32 μL of sterile distilled water. Amplification of these markers used a GeneQ PCR Thermal Cycler with the following thermal profile; 35 cycles of 90 sec at 94 °C, 60 sec at 58 °C, and 60 sec at 72 °C. Amplicons were checked on 1% agarose gel electrophoresis. All PCR products were treated to remove excess primers and dNTP using Exonuclease I (TaKaRa) and Shrimp Alkaline Phosphatase (TaKaRa). DNA sequences were determined by ABI3000 using a research contract service (Ltd.
FASMAC). DNA sequences of 709 bases for \textit{mtMutS}, 804 for \textit{COI}, 773 for \textit{28S} rDNA, and 673 for \textit{ND2} were obtained in this study. DNA sequences for \textit{mtMutS}, \textit{COI}, and \textit{28S} were combined and analyzed because concatenated DNA sequences using these markers have been recently used for the molecular phylogenetic analyses in the Xeniidae (McFadden et al. 2019; Halász et al. 2019), while sequences for \textit{ND2} were analyzed alone because of restricted number of sequences available (McFadden et al. 2006; McFadden and Ofwegen 2012; McFadden et al. 2014b; McFadden et al. 2017). As outgroups for both analyses, we used \textit{Paralemnalia thyroides} (Ehrenberg, 1834) (family Nephtheidae), \textit{Rhytisma fulvum} (Forskål, 1775) (family Alcyoniidae) and \textit{Coelogorgia palmosa} Milne Edwards & Haime, 1857 (family Coelogorgiidae), which are all known to be closely related to the Xeniidae (Halász et al. 2019). MEGA6 (Tamura et al. 2013) was used to select appropriate models (T92+G model for the concatenated DNA sequences, including \textit{mtMutS}, \textit{COI}, and \textit{28S}, and T92 model for \textit{ND2}) for maximum likelihood (ML) method and to reconstruct the ML phylogenetic trees with 1000 bootstrap replicates. In Bayesian analysis, the concatenated alignment data was treated as a separate data partition with different models of evolution applied to each of the mitochondrial (\textit{mtMutS} and \textit{COI}: HKY+G) and nuclear (\textit{28S}: GTR+G) markers. MrBayes v. 3.2.1 (Ronquist et al. 2012) was run for 50,000,000 generations (until standard deviation of split partitions < 0.01) with a burn-in of 25% and default Metropolis coupling parameters. For phylogenetic analyses, recently published data for three markers (\textit{mtMutS}, \textit{COI}, and \textit{28S}) from the Xeniidae were also added (Table 1).

\textbf{Table 1.} List of specimens of the family Xeniidae examined in this study and accession numbers for \textit{28S}, \textit{mtMutS}, \textit{COI} and \textit{ND2} markers. The origin of the accession number is shown by asterisk (s) in the reference list for each line if more than one reference exists.

| Species | Specimen Catalog # | GenBank accession number | References |
|---------|--------------------|--------------------------|------------|
| *Xenia konohana* sp. nov. | KBF-OA–00092 | LC656679* LC656674* LC656676* LC467035** | *This study |
| *Xenia konohana* sp. nov. | KBF-OA–00093 | LC656680* LC656673* LC656677* LC467036** | *This study |
| *Xenia konohana* sp. nov. | KBF-OA–00094 | LC656681* LC656675* LC656678* LC467037** | *This study |
| *Anthelia glauca* | ZMTAU CO34183 | JX203753* JX203812* GQ342460** | *McFadden and Ofwegen 2012, **Brockman and McFadden 2012 |
| *Asterospicularia laurae* | CSM-OCN8971L | KM201433 KM201452 KM201458 | – |
| *Asterospicularia randalli* | RMNH:Coel. 41521 | KF915316 KF915556 KF955019 | – |
| *Heteroxenia mindorensis* | CAS:IZ:184566 | KJ511300 KJ511339 KJ511379 KJ511421 | McFadden et al. 2014a |
| *Heteroxenia mindorensis* | CAS:IZ:184574 | KJ511381 KJ511341 KJ511302 KJ511423 | McFadden et al. 2014b |
| *Ovabunda ainex* | ZMTAU:36785 | KY442364 KY442323 KY442342 KY442395 | McFadden et al. 2017 |
| *Ovabunda ainex* | ZMTAU:36786 | KY442365 KY442324 KY442343 KY442396 | McFadden et al. 2017 |
| *Ovabunda andamanensis* | PMBBC:11861 | KM201440 KM201455 KM201461 | – |
| *Ovabunda andamanensis* | PMBBC:11862 | KM201439 KM201454 KM201460 | – |
| Species                | Specimen Catalog # | GenBank accession number | References |
|-----------------------|-------------------|--------------------------|------------|
| Ovabunda biseriata    | ZMTAU:34876       | KY442376 KY442330 KY442349 KY442405 | McFadden et al. 2017 |
| Ovabunda biseriata    | ZMTAU:34881       | KY442378 KY442332 KY442351 KY442407 | McFadden et al. 2017 |
| Ovabunda biseriata    | ZMTAU:34882       | KY442379 KY442333 KY442352 KY442408 | McFadden et al. 2017 |
| Ovabunda faraunenesis | ZMTAU:CO 34051    | KJ511306* GU356029* GU356006* KJ511427** | *McFadden et al. 2011, **McFadden et al. 2014b |
| Ovabunda faraunenesis | ZMTAU:34884       | KY442380 KY442334 KY442353 KY442412 | McFadden et al. 2017 |
| Ovabunda faraunenesis | ZMTAU:34886       | KY442381 KY442335 KY442354 KY442413 | McFadden et al. 2017 |
| Ovabunda impulstilla   | ZMTAU:34571       | KY442374 KY442328 KY442347 KY442418 | McFadden et al. 2017 |
| Ovabunda impulstilla   | ZMTAU:34891       | KY442383 KY442357 KY442356 KY442419 | McFadden et al. 2017 |
| Ovabunda obscuronata   | ZMTAU:CO 34077    | KJ511307* GU356027* GU356004* KJ511428** | *McFadden et al. 2011, **McFadden et al. 2014b |
| Sansibia flav a        | ZMTAU:Co36004     | MK400137 MK396681 MK396728 – | McFadden et al. 2019 |
| Sansibia flav a        | ZMTAU:Co36006     | MK030486 MK030380 MK039204 – | McFadden et al. 2019 |
| Sansibia flav a        | ZMTAU:Co36073     | MK030487 MK030381 MK039205 – | McFadden et al. 2019 |
| Symposi dium caeruleum| ZMTAU CO34185     | JX203758* JX203815* GU356009** KJ511430*** | *McFadden and Ofwegen 2012, **McFadden et al. 2014b, ***McFadden et al. 2017 |
| Xenia fisheri          | CAS:IZ:184540     | KJ511311 KJ511349 KJ511389 KJ511436 | McFadden et al. 2014b |
| Xenia fisheri          | CAS:IZ:184541     | KJ511312 KJ511350 KJ511390 KJ511437 | McFadden et al. 2014b |
| Xenia kunimotoensis   | CAS:IZ:184554     | KJ511314 KJ511352 KJ511392 KJ511441 | McFadden et al. 2014b |
| Xenia lepida          | CAS:IZ:184535     | KJ511316 KJ511354 KJ511394 KJ511443 | McFadden et al. 2014b |
| Xenia lepida          | CAS:IZ:184562     | KJ511317 KJ511355 KJ511395 KJ511444 | McFadden et al. 2014b |
| Xenia membranacea     | CAS:IZ:184536     | KJ511308 KJ511345 KJ511385 KJ511432 | McFadden et al. 2014b |
| Xenia membranacea     | CAS:IZ:184548     | KJ511319 KJ511357 KJ511397 KJ511446 | McFadden et al. 2014b |
| Xenia membranacea     | CAS:IZ:184549     | KJ511320 KJ511358 KJ511398 KJ511447 | McFadden et al. 2014b |
| Xenia puertogalerae   | CAS:IZ:184532     | KJ511324 KJ511362 KJ511402 KJ511451 | McFadden et al. 2014b |
| Xenia puertogalerae   | CAS:IZ:184539     | KJ511325 KJ511363 KJ511403 KJ511452 | McFadden et al. 2014b |
| Xenia puertogalerae   | CAS:IZ:184545     | KJ511326 KJ511364 KJ511404 KJ511453 | McFadden et al. 2014b |
| Xenia viridis         | CAS:IZ:184542     | KJ511331 KJ511369 KJ511409 KJ511458 | McFadden et al. 2014b |
| Xenia hicksoni        | ZMTAU CO34072     | JX203759* GQ342529** GQ342463** KJ511438* | *McFadden and Ofwegen 2012, **Brockman and McFadden 2012 |
| Xenia ternatana       | CAS:IZ:184560     | KJ511327 KJ511365* KJ511405* KJ511454 | McFadden et al. 2014b |
| Xenia umbellata       | ZMTAU:36783       | KY442362* KT590452** KT590435** KY442431* | *McFadden et al. 2017, **Halász et al. 2019 |
| Xenia umbellata       | ZMTAU:36788       | KY442367* KT590457** KT590438** KY442432* | *McFadden et al. 2017, **Halász et al. 2019 |
| Xenia umbellata       | ZMTAU:36790       | KY442369* KT590458** KT590439** – | *McFadden et al. 2017, **Halász et al. 2019 |
| Yamazatusm inbatum     | ZMTAU:Co35143     | MH071864 MK030449 MK039274 – | McFadden et al. 2019 |
| Yamazatusm inbatum     | ZMTAU:Co35144     | MH071865 MH071910 MH071958 – | Benyahu et al. 2018a |
| Yamazatusm inbatum     | ZMTAU:Co35741     | MK030452 MK030451 MH071955 – | McFadden et al. 2019 |
| Unomia stolonifera     | ZMTAU Co38081     | MT489336 MT482554 MT487559 | Benyahu et al. 2021 |
| Coelogorgia palmosa    | NTM C14914        | JX203698 DQ302805 GQ342413 DQ302879 | McFadden et al. 2006 |
| Rhytisma fulvum        | ZMTAU CO34124     | JX203728* GQ342478** GQ342396** – | *McFadden and Ofwegen 2012, **Brockman and McFadden 2012 |
| Paralemnalia thyroidea | ZMTAU:Co36976     | MHS16907 MHS16632 MHS16518 – | Benyahu et al. 2018b |
| Cladiella digitulata   | MUFSCOSU14        | – – – LC467083 | Koido et al. 2019 |
| Cladiella pharephora   | MUFSCOAK1         | – – – LC467084 | Koido et al. 2019 |
| Kyzyum sp.             | MUFSCOMO150       | – – – LC467086 | Koido et al. 2019 |
| Kyzyum sp.             | MUFSCOMO164       | – – – LC467087 | Koido et al. 2019 |
| Kyzyum sp.             | MUFSCOOTUD8       | – – – LC467088 | Koido et al. 2019 |
Results

Taxonomy

Class Anthozoa Ehrenberg, 1831
Subclass Octocorallia Haeckel, 1866
Order Alcyonacea Lamouroux, 1812
Family Xeniidae Ehrenberg, 1828

Genus *Xenia* Lamarck, 1816

Type species. *Xenia umbellata* Lamarck, 1816

*Emended diagnosis.* (Chiefly after Halász et al. 2019). Colonies are small and soft with cylindrical stalk, undivided or branched, terminating in one or more domed polyp-bearing regions. Polyps are not retractile and are always monomorphic. The dominant sclerites are ellipsoid platelets, usually abundant in all parts of the colony. They are composed of calcite rods, often dendritic or sinuous, mostly radially arranged, at least at the periphery of the sclerites. In addition to ellipsoid platelets, a few species have rods or unique spindles with pointed spear ends.

*Xenia konohana* sp. nov.

http://zoobank.org/D1BD260D-A55D-4A88-9CF6-823E06AF0504

New Japanese name: konohana-umiazami

Figs 3–10

Synonym. *Xenia* sp. 1 Koido et al. 2019: Table 1, figs 2J–4J.

Materials. **Holotype**: KBF-OA-00092 (MUFS-COMO4 in Koido et al. 2019), Oshima Isl., Nichinan City, Miyazaki Prefecture, depth < 5 m, July 2, 2012. **Paratypes**: KBF-OA-00093 (MUFS-COMO53 in Koido et al. 2019), Oshima Isl., Nichinan City, Miyazaki Prefecture, depth < 10 m, December 25, 2012; KBF-OA-00094 (One colony with two stems) (MUFS-COMO54 in Koido et al. 2019), Oshima Isl., Nichinan City, Miyazaki Prefecture, depth < 10 m, December 25, 2012.

**Descriptions.** The holotype (Fig. 2A) displays a typical *Xenia*-style growth form (Alderslade 2001; Benayahu 2010), featuring a distinct cylindrical stalk, 35 mm high and 20 mm wide attached to a rock. The colony possesses three branches 5–7 mm long from a common basal stalk. The whole colony is creamy white in ethanol. Polyps are 4.5–5.0 mm long, excluding tentacles, and 2.0 mm in diameter at their proximal part. Tentacles are 3.0–4.0 mm long and 0.3–0.5 mm wide at their proximal part.

Pinnules are arranged mostly in three rows along each side of the tentacles, leaving free median space along the oral side. This space is not always visible at the distal part of the longest tentacles. The number of rows of pinnules drops to two toward the proximal part of the tentacle, and occasionally, only a single row can be seen (Fig. 3).
Figure 2. Fixed specimens of *Xenia konohana* sp. nov. A holotype BF-OA-00092 B paratype KBF-OA-00093 C, D paratype KBF-OA-00094. Scale bar: 10 mm.
Figure 3. Tentacles of *Xenia konohana* sp. nov. aboral (left) and oral sides (right) A schema of holotype KBF-OA-00092: three rows (the number is shown in the upper-right) and 13 pinnules at the outermost row (the number is shown in the center) B holotype KBF-OA-00092 C paratype KBF-OA-00093 D paratype KBF-OA-00094. Scale bar: 1 mm.
The outermost row usually includes 12–16 pinnules each, up to 0.23 mm long and 0.21 mm wide at the proximal part. Typically, no gap between pinnules exists, but in rare cases, a gap of approximately 0.05 mm is observed.

Sclerites are abundant in polyps and surface layers of stalk and branches but absent interior. Under light microscopy, two forms of sclerites are observed – simple platelets (Fig. 4A) and spindles (Fig. 4B). Platelets are brown-red and spindles transparent (Fig. 4) under transmitted illumination. Platelets look pale blue and spindles appear transparent under epi-illumination (Fig. 5).

**Polyp sclerites.** Two forms of sclerites, simple platelets and spindles, are seen in polyps (Figs 6A, B, 7A, B). Simple platelets are 0.016–0.021 mm long and 0.009–0.011 mm wide. Spindles, 0.035–0.049 mm long and 0.004–0.006 mm wide, display unique ends with pointed spear tips. Sclerite composition in tentacles (n = 124) is 7.3% simple platelets and 92.7% spindles. In the polyp body (n = 83), these proportions are 4.8% and 95.2%, respectively. Thus, the vast majority of sclerites are spindles. Some spindles have thorns on their surface.

**Stalk and branch sclerites.** Two forms of sclerites, simple platelets and spindles, are also found in stalk and branches (Figs 6C, D, 7C, D). Simple platelets, several with an indistinct median waist, are 0.017–0.021 mm long and 0.009–0.011 mm wide. Spindles are 0.038–0.049 mm long and 0.004–0.006 mm wide. All spindles are more or less bent. Sclerite composition in stalk (n = 104) is 7.7% simple platelets and 92.3% spindles. Thus, the vast majority of sclerites are spindles.

![Figure 4. Light microscope images of sclerites in polyps of *Xenia konohana* sp. nov., holotype KBF-OA-00092](image) A spindles B simple platelets.
Microstructure of sclerites. The platelets are composed of branched sinuous dendritic rods within the sclerite interior. SEM at 30,000–50,000× magnification shows distal parts of rods that line up almost vertically and parallel to the surface (Fig. 8A, B). The spindles are composed of fused grains with a granular appearance (Fig. 8C, D). Fused grains also exist inside, which can be observed in cross-sections of broken spindles (Fig. 8E, F). Both ends of the spindles are relatively smooth (Fig. 8G). Thorns may form on the surface of spindles (Fig. 7, red arrows indicate the thorn, Fig. 8D shows the thorn expansion).

Variation. Two preserved paratypes (KBF-OA-00093, KBF-OA-00094) differ in size (Fig. 2B, C). Both paratypes are smaller than the holotype (30 mm high, 15 mm wide of KBF-OA-00093, and 9–16 mm high, 6–9 mm wide of KBF-OA-00094). One paratype (KBF-OA-00094) does not branch but has two stalks connected at the bottom, although this specimen, accidentally, is broken into two pieces (Fig. 2C, D). Tentacle size is 4.0 mm long and 0.5 mm wide for KBF-OA-00093 and 3.0 mm long and 0.5 mm wide for KBF-OA-00094 (Fig. 3C, D). Paratypes display three rows of pinnules along each side of tentacles, consistent with the holotype. Pinnule numbers in the outermost row are 13–16 for KBF-OA-00093, and 12–14 for KBF-OA-00094, compared to 12–16 for the holotype. All paratypes have the two forms of sclerites as well as holotype (Fig. 9, 10), and are similar in the composition. In all parts of all specimens, the vast majority of sclerites are spindles, with the percentages being approximately 83–94% (Table 2).
Xenia konohana sp. nov., a new soft coral species from Miyazaki, Japan

Locality. The species is common in waters around Oshima Island, Miyazaki, Japan, at depths from 5 to 10 m. Specimens exist attached to the surface of rocks or rock debris.

Etymology. Konohana is named after a goddess in Japanese mythology, “Kono-hanasakuya-hime” (“hime” is “princess” in English). Her shrine is in Miyazaki Prefecture. The present study also proposes a standard Japanese name “konohana-umiazami” for X. konohana sp. nov. The specimen KBF-OA-00092 is designated as the standard specimen for this new Japanese name.

Remarks. Most Xenia species have only ellipsoid platelets or spheroid sclerites (Halász et al. 2019). Although only two species, X. membranacea Schenk, 1896 and X. depressa Kükenthal, 1909 have been reported to display rod-shaped sclerites in their original descriptions, this type of sclerite has not been found in the syntype of X. membranacea (Halász et al. 2019), and X. depressa has never been re-described and the existence of the type materials are unknown. Therefore, we treated the existence of rod-shaped sclerites as either incorrect for X. membranacea or unverified for X. depressa in this study. On the other hand, X. konohana sp. nov. (= Xenia sp. 1 by Koido et al. 2019) has unique spindle sclerites in addition to ellipsoid platelets (Figs 4–10). This combination does not occur in other species in the genus. Moreover, it is clear that spindles are the majority sclerites in tentacles, polyp body and stalks for all three specimens (KBF-OA-00092 to KBF-OA-00094).

All three specimens (KBF-OA-00092 to KBF-OA-00094) were nearly identical in sclerite shape, size and composition of two types of sclerite forms (xeniid platelets and unique spindles), number of pinnules, and molecular phylogenetic position. Eight species of Xenia (X. blumi Schenk, 1896, X. crassa Schenk, 1896, X. cylindrica Roxas 1933, X. fisheri Roxas, 1933, X. garciae Bourne, 1895, X. hicksoni Ashworth, 1899, X. ternatana Schenk, 1896, and X. viridis Schenk, 1896), which partly overlap with X. konohana sp. nov. in exhibiting platelet sclerites, 3–4 rows of pinnules and 12–23 outermost row of pinnules, are distinguishable by the absence of the specific sclerite form, “unique spindle” (Table 3). A variation of pinnules has been reported in many species in xeniid genera, and the number of pinnules is likely to be unreliable as a character to determine the species boundaries (Halász et al. 2019; McFadden et al. 2017). Therefore, the information on sclerites is more important than ever as a character for identifying species boundaries.

### Table 2. Sclerite composition of Xenia konohana sp. nov.

|                | Tentacles |          | Polyp body |          | Stalk |          |
|----------------|-----------|----------|------------|----------|-------|----------|
|                | platelets | spindles | platelets  | spindles | platelets | spindles |
| KBF-OA-00092 (holotype) | Fig. 2A    | n = 124  | n = 83     | n = 104  | 7.3%  | 92.7%    |
|                |           | 92.7%    | 4.8%       | 95.2%    | 7.7%  | 92.3%    |
| KBF-OA-00093 (paratype) | Fig. 2B    | n = 123  | n = 132    | n = 85   | 5.7%  | 94.3%    |
|                |           | 94.3%    | 10.6%      | 89.4%    | 7.1%  | 92.9%    |
| KBF-OA-00094 (paratype) | Fig. 2C    | n = 138  | n = 103    | n = 91   | 10.1% | 89.9%    |
|                |           | 89.9%    | 5.8%       | 94.2%    | 6.6%  | 93.4%    |
|                | Fig. 2D   | n = 92   | n = 152    | n = 96   | 12.0% | 88.0%    |
|                |           | 88.0%    | 17.1%      | 82.9%    | 7.3%  | 92.7%    |
Figure 6. Scanning electron micrographs of platelets of *Xenia konohana* sp. nov., holotype KBF-OA-0009
A in tentacles B in polyp body C in stalk surface D in branch surface. Scale bar: 0.010 mm.
Molecular phylogenetic results

Molecular phylogenetic trees using the ML and Bayes methods showed very similar topologies. Therefore, in this study, only ML trees are shown (Figs 11, 12). As pointed out in previous studies (Halász et al. 2019; Benayahu et al. 2021), the genus Xenia is paraphyletic and polyphyletic with some other taxa, and separated into three clades (clades X1–X3) in the mtMutS+COI+28S tree (Fig. 11). All three clades were supported by high bootstrap values (75 to 99%) and posterior probabilities (1). Asides from Xenia, clade X1 included...
Ovabunda, clade X2 included Heteroxenia, and clade X3 included Sansibia, Yamazatum and Unomia. All three specimens of X. konohana sp. nov., which had the same DNA sequences for all four markers, belonged to clade X1 forming a sister clade with Ovabunda spp., X. umbellata and X. hicksoni, and united with X. lepida Verseveldt, 1971 and X. viridis within a strongly supported subclade (bootstrap values: 95%, posterior probability: 1).

Figure 7. Scanning electron micrographs of spindles of Xenia konohana sp. nov., holotype KBF-OA-00092 A in tentacles B in polyp body C in stalk surface D in branch surface. Arrow indicates thorns on the surface of spindles. Scale bar: 0.010 mm.
**Figure 8.** Scanning electron micrographs of the surface of sclerites in tentacles of *Xenia konohana* sp. nov., holotype KBF-OA-00092. **A** surface of platelets covered by minute papillae. **B** broken platelets with radial dendritic rods. **C** central surface of spindle covered by minute granular. **D** thorns on the surface of spindles. **E** broken spindle. **F** close-up view of a broken spindle with fused grain. **G** tip of a spindle. Scale bar: 0.001 mm.
Figure 9. Scanning electron micrographs of paratype (KBF-OA-00093) of *Xenia konohana* sp. nov.: A platelets B spindles (arrow indicates thorns on the surface of spindles) C surface of platelets D central surface of spindle E tip surface of a spindle F thorns on the surface of spindles. Scale bar: 0.01 mm (A, B); 0.001 mm (C–F).
Figure 10. Scanning electron micrographs of paratype (KBF-OA-00094) of *Xenia konohana* sp. nov.: A platelets B spindles (arrow indicates thorns on the surface of spindles) C surface of platelets D central surface of spindle E tip surface of a spindle F thorns on the surface of spindles. Scale bar: 0.01 mm (A, B); 0.001 mm (C–F).
On the other hand, in the ND2 tree, *Xenia* was separated into only two clades (XN1 and XN2) (Fig. 12). Clade XN1 was strongly supported by high bootstrap value (100%) and posterior probability (1), and included the same members with all three specimens of *X. konohana* sp. nov. in clade X1 in the *mtMutS*+COI+28S tree. For clade XN2, this clade was not supported by bootstrap values and posterior probabilities, but three *Xenia* species and *Heteroxenia mindorensis* in this clade were genetically identical. Clade XN2 included members belonging to both clades X2 and X3 in the *mtMutS*+COI+28S tree.

Although *X. viridis* was not genetically separated from *X. konohana* sp. nov. in the ND2 tree (Fig. 12), they were clearly separated from each other in the *mtMutS*+COI+28S tree (Fig. 11). Thus, the molecular phylogenetic tree based on the concatenated DNA sequences of *mtMutS*, COI, and 28S, and the tree based on ND2 support the phylogenetic position of *X. konohana* sp. nov. in the genus *Xenia* (Figs 11, 12).

**Figure 11.** Phylogenetic relationships of species in the Xeniidae based on the concatenated *mtMutS*, COI and 28S sequences. Numbers above main branches show percentages of bootstrap values (> 50%) in maximum likelihood analysis; numbers below main branches show Bayesian posterior probabilities. X1, X2 and X3 denote clades defined by McFadden et al. (2014b). *Xenia konohana* sp. nov. is shown in red.

**Figure 12.** Phylogenetic relationships of species in the Xeniidae based on ND2 sequences. Numbers above main branches show percentages of bootstrap values (> 50%) in maximum likelihood analysis; numbers below main branches show Bayesian posterior probabilities. *Xenia konohana* sp. nov. is shown in red.
Discussion

The genus *Xenia* is polyphyletic and paraphyletic with other xeniid genera such as *Ovabunda*, *Heteroxenia*, *Sansibia*, *Asterospicularia*, *Unomia*, and *Yamazatum* based on molecular studies (Janes et al. 2014; McFadden et al. 2014b; Benayahu et al. 2018a; Halász et al. 2019; Benayahu et al. 2021). In the present study, *Xenia* was also polyphyletic as well as paraphyletic with some other genera (Figs 11, 12), but *X. konohana* sp. nov. formed a clade with two congeneric species, *X. lepida* and *X. viridis*, and was closely related to a sister clade with *Ovabunda* spp., *X. hicksoni* and *X. umbellata*. These four *Xenia* species are similar to *X. konohana* sp. nov. in the number of rows and the outermost row of pinnules, but they do not exhibit spindle sclerites. *Ovabunda* exhibits only simple platelets like *Xenia*, but it also displays a corpuscular surface microstructure on platelet surfaces. *Xenia*, including *X. konohana* sp. nov., exhibits a dendritic microstructure on these surfaces of simple platelets. Further taxonomic revision of *Xenia* and related genera such as *Ovabunda*, *Heteroxenia*, *Sansibia*, *Asterospicularia*, *Unomia*, and *Yamazatum* may be necessary due to these phylogenetic relationships. Still, we conclude that *Xenia konohana* sp. nov. is a new member of *Xenia* based on molecular phylogenetic relationships and the presence of unique spindles along with *Xenia*-specific ellipsoid platelets with dendritic surface microstructure.

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