The taxonomic challenge posed by the Antarctic echinoids Abatus bidens and Abatus cavernosus (Schizasteridae, Echinoidea).

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Abstract

Cryptic species have been repeatedly described for two decades among the Antarctic fauna, challenging the classic model of Antarctic species with circumpolar distributions and leading to revisit the richness of the Antarctic fauna. No cryptic species had been so far recorded among Antarctic echinoids, which are, however, relatively well-diversified in the Southern Ocean. The R/V *Polarstern* cruise PS81 (ANT XXIX/3) came across populations of *Abatus bidens*, a schizasterid so far known by few specimens that were found living in sympatry with the species *Abatus cavernosus*. The species *A. cavernosus* is reported to have a circum-Antarctic distribution, while *A. bidens* is only recorded with certainty in South Georgia and at the northern tip of the Antarctic Peninsula. Based on genetic and morphological analyses, our results clearly show that *A. bidens* and *A. cavernosus* are two distinct species. The analyzed specimens of *A. bidens* group together in two haplogroups separated from one another by 2.7% of genetic difference. They are located in the Weddell Sea and in the Bransfield Strait. Specimens of *A. cavernosus* form one single haplogroup separated from haplogroups of *A. bidens* by 5% and 3.5% of genetic difference respectively. The species was collected in the Drake Passage and in the Bransfield Strait. Morphological analyses allow to differentiate *A. bidens* from *A. cavernosus*. In contrast, the two genetic groups of *A. bidens* cannot be differentiated from one another based on morphology alone, suggesting that they could represent a case of cryptic species, common in many Antarctic taxa, but not yet reported in Antarctic echinoids. This need to be confirmed by complementary analyses of independent genetic markers.

Keywords: *Abatus bidens*, *Abatus cavernosus*, cryptic species, Echinoidea, Schizasteridae, Southern Ocean

Introduction

For two decades, cryptic species have been repeatedly described among the Antarctic fauna, challenging the former, classic biogeographic model of Antarctic species with wide, circumpolar distributions (De Broyer et al. 2011; Dettai et al. 2011). The richness and spatial distribution of the Antarctic fauna have been revisited and the underpinning evolutionary processes re-assessed (Kaiser et al. 2013). The occurrence of cryptic species in the Southern Ocean, and on the Antarctic continental shelf in particular was highlighted in many studies of population genetics, in some cases even signing the existence of species flocks (Lecointre et al. 2013). Cryptic species have been described in various Antarctic groups: polychaetes (Schüller 2011), gastropods (Wilson et al. 2013), bivalves (Linse et al. 2007), crustaceans (Held and Wägele 2005; Raupach and Wägele 2006), pycnogonids (Krabbe et al. 2009), cephalopods (Allcock et al. 2010), crinoids (Wilson et al. 2007; Hemery et al. 2012), holothuroids (O'Loughlin et al. 2011), and fish (Smith et al. 2011). Whatever the clade considered, depending on historical and environmental contingencies, the processes leading to cryptic speciation can be related to spatial isolation, disruptive selection, and ecological divergence. Cryptic species being recurrently described in various Antarctic clades, its origin must be soughed for in mechanisms impacting the entire Antarctic fauna and linked to relatively fast landscape dynamics that is, rapid alternation between allopatry and sympatry (Aguilée et al. 2012). A common explanation resides in the alternation between glacial and interglacial periods that forces populations to isolated, glacial refugia or otherwise allows species to expand their spatial ranges (Thatje et al. 2005; Allcock and Strugnell 2012; Aguilée et al. 2012).

Unexpectedly, no cryptic species had been so far recorded among Antarctic echinoids, which are, however, relatively well-diversified in the Southern Ocean (Saucède et al. 2014). Spatangoid irregular echinoids in particular form the most speciose group of echinoids in the Southern Ocean. It is exclusively represented there by the family Schizasteridae and counts 30 morphologically recognized species and eight genera (David et al. 2005a, b; Pierrat et al. 2012, Saucède et al. 2014). Most species are brooders, the females incubating their young in deepened petals that form kind of pouches or marsupia. Such a life history trait implies limited dispersal capabilities that might have facilitated this outstanding diversification of the group also promoted by the alternation between glacial and interglacial periods and the flow of the Antarctic Circumpolar Current (Poulin et al. 2002; Pearse et al. 2009). All Antarctic species of Schizasteridae constitute a single monophyletic grouping, which presumably represent a species "core flock" (*sensu* Lecointre et al. 2013) endemic to the Antarctic continental shelf including its deepest parts (David et al. 2005a; Pearse et al. 2009; Lecointre et al. 2013; Saucède et al. 2014). Schizasterids are deposit feeders and soft-bottom dwellers, they live more or less buried into the superficial layer of sediments.

The systematics of Antarctic Schizasteridae has long been explored based on morphological observations (e.g. Mortensen 1909, 1910; Koehler 1912; Pawson 1969; David et al. 2005a, b for the most recent reviews) but several species are known by few specimens and still remain poorly described. Considering the significant variation of morphological characters such as test outline and plating, development and position of fasciolar bands and of other appendages, the taxonomy of Antarctic Schizasteridae and the phylogenetic relationships among species remain puzzling, pending for new appraisals grounded on molecular markers. The R/V *Polarstern* cruise PS81 - ANT XXIX/3 (Gutt 2013) came across populations of *Abatus bidens*, a schizasterid so far known by few specimens that were found living in sympatry with the species *Abatus cavernosus*. The species were formerly considered as two

varieties of a same species (Mortensen 1951) before they were placed in two distinct entities (David et al. 2005a). The species *A. cavernosus* is so far reported to have a circum-antarctic distribution, while *A. bidens* is only recorded with certainty in South Georgia and at the northern tip of the Antarctic Peninsula. The later species was also reported in Adelie Land where its occurrence remains uncertain (David et al. 2005a). The present study makes possible to fully describe the species *A. bidens* for the first time and to assess morphological and genetic variations between the two forms. What are the morphological and genetic differences between *A. bidens* and *A. cavernosus* ? Are there reliable, genetic differences to support the distinction between the two nominal species ? Are there transitional forms to suggest that they are cryptic species if any?

Materials and methods

Material studied

Seventy nine specimens of *A. bidens* and *A. cavernosus* were collected at 19 stations in the northwest of the Weddell Sea, in the Bransfield Strait, and in the Drake Passage during cruise PS81 of the R/V *Polarstern* (Fig. 1; Table 1). Specimens were collected between 102 and 782 m depth using an Agassiz trawl (Gutt 2013). The two species were found in sympatry at five of the 19 stations. Specimens were subsequently fixed in 96% ethanol and are now housed in collections of the University of Burgundy (Dijon, France). Taxonomy of Antarctic species of the family Schizasteridae is really challenging as shown for long by previous studies (David et al. 2005a, Saucède et al. 2014). We therefore concentrated our study on recently sampled specimens during cruise PS81, for which a special care was taken to control sample preservation, morphological study, and taxonomic identification. Genetic and morphometric analyses were performed independently.

Genetic analyses

Genetic analyses initially included 62 specimens among which 49 provided informative sequences to be analyzed. Tissue samples were taken on echinoid spines and DNA was extracted using the Chelex method following Chenuil and Féral (2003). A typical barcoding region of the mitochondrial cytochrome oxidase gene was amplified by PCR using the primers COIe3 (5'- GCTCGTGC(A/G)TCTAC(A/G)TCCAT -3') and COIe5 (5'- GC(C/T)TGAGC(A/T)GGCATGGTAGG -3') from Stockley et al. (2005). PCR amplicons were sent to the industry (Eurofins Genomics) for Sanger sequencing. DNA sequences were manually trimmed and checked using the program Bioedit (Hall 1999). Haplotype networks were built by the median-joining method using the Network software (Bandelt et al. 1999). A color code was used to identify the morphological species, the distribution area (inside the Weddell Sea, the Bransfield Strait, and the Drake Passage), and the sampling stations of each haplotype owing to the NetworkPublisher software (Bandelt et al. 1999).

Morphological analyses

Echinoids were observed under a binocular microscope and morphology was analyzed using 10 quantitative parameters measured on echinoid tests with a digital caliper to the nearest 0.1 mm. These measurements were defined based on the morphological characters described in diagnoses of Antarctic echinoid species (David et al. 2005a). They are total test length (LL), total test width (WD), total test height (HT), distance between the apical system and the back of the test (AS), length of posterior petal I (PPT), length of anterior petal II (APT), distance between center of

the apical system and beginning of petal II (DP), distance between the periproct and the base of the test (PP), distance between the peristome and the back of the test (PS), and total number of adjacent ambulacral plates abuting the labrum (LB) (Fig. 2). Echinoid size was estimated using the geometric mean of test length, width and height (i.e., the cube root of their product) and test shape (i.e., test proportions) was analyzed using seven indices computed from ratios between some of the 10 measured quantitative parameters: shape at the ambitus (AMB=WD/LL), gibbosity (GIB=HT/[√(LL.WD)]), relative position of the peristome (RPS=PS/LL), relative position of the periproct (RPP=PP/HT), relative position of the apical system (RAS=AS/LL), relative size of the petals (RPET=PPT/APT), relative importance of the gap between the apical system and the beginning of petal II (RDP=DP/APT). Morphometric analyses were performed on 75 specimens, 57 identified as *A. bidens* and 18 as *A. cavernosus*, using the softwares Past (Hammer et al. 2001) and Statistica 6.1 (Statsoft 2002). Principal component analyses (PCAs) were performed independently on the 10 quantitative parameters and on the seven indices to analyze morphological variations within and between the two species. Correlation matrices were used to compute the PCAs as raw data show contrasting units and range variations. Morphological differentiation between the two species was analyzed using discriminant analyses based on quantitative measurements and on the indices independently. A priori groups were determined based on genetic data (see below). These analyses were complemented by parametric tests as homoscedasticity assumptions are respected (only parameters PP and LB and indices RPS and RPP show significantly different variances between the two species).

Appendages were prepared and examined following David and Mooi (1990). Pedicellariae were removed from tests and placed into 96 % ethanol. They were bleached with a 10 % solution of sodium hypochlorite to remove soft tissue and to separate the valves. Then, they were washed in water, dried for 24 h, and mounted on SEM stubs. Details of pedicellariae were examined with a tabletop scanning electron microscope, and digital images were recorded.

Results

Genetic analyses

After trimming, the alignment file contained 633 homologous base pairs for 49 individuals. Seven haplotypes were identified and form three divergent haplogroups, each group including two or three haplotypes (Fig. 3). Haplogroups G1 (26 specimens) and G2 (18 specimens) are composed of specimens of *A. bidens* and are separated from one another by a minimum of 17 mutations (i.e., 2.7% of genetic difference). The third haplogroup G3 (5 specimens) is composed of specimens of *A. cavernosus* only, and is separated from haplogroups G1 and G2 by a minimum of 31 and 23 mutations respectively (i.e., 5% and 3.5% of genetic difference) (Fig. 3a). Haplogroups were identified at 11 of the 19 sampling stations. Haplogroup G3 was identified at stations of the Drake Passage and in the Bransfield Strait, while the two divergent haplogroups of *A. bidens* were found at stations of the Weddell Sea and of the Bransfield Strait (Fig 3b). The three haplogroups occur in sympatry at two stations only (Sts. 220-2 and 224-3), the two haplogroups of *A. bidens*, G1 and G2, are present together in 3 other stations (Sts. 164-4, 204-2, and 227-2), one single haplotype was identified at the six remaining stations (Fig. 3c).

Morphological analyses

Within the 75 specimens measured, 41 were identified as *A. bidens* by genetic analyses, 26 belong to haplogroup G1, 15 to haplogroup G2, and 5 more specimens were identified as *A. cavernosus* and belong to haplogroup G3. A total of 29 specimens could not be analyzed for genetics and were finally attributed to their respective species based on morphological results that is, 16 specimens to *A. bidens* and 13 to *A. cavernosus*. The distribution of specimens is detailed in Table 1.

Statistical analyses of morphological data allow to clearly differentiate the two morphological species *A. bidens* and *A. cavernosus.* Five parameters, in particular, were tested significantly different between the two species (Student bilateral tests are detailed in Table 2): the distance between the peristome and the back of the test (PS), the distance between the apical system and the back of the test (AS), the length of the posterior petal I (PPT), the distance between the center of the apical system and the beginning of petal II (DP), and the total number of adjacent ambulacral plates abuting the labrum (LB). Size was not tested significant between the two species (bilateral t-test under homoscedasticity: p=0.47) so that morphological differences between species are not due to allometry. Sexual dimorphism was not tested significant either for shape differences (Hotelling's T-squared: p=0.19 and p=0.29 for raw parameters and shape indices respectively). Size only differs between females and males, females being larger than males (unilateral *t*-test: p=0.014, mean size of females and males: 38.5 mm and 35.4 mm respectively).

The principal component analysis performed on the 10 quantitative parameters (Fig. 4) shows that the two species mainly differentiate from one another along PC2 (14.2% of the total variance), which is mainly due to three parameters: labrum extension (LB), length of posterior petal I (PPT), and position of the apical system (AS). The principal component analysis performed on shape indices gave congruent results with a clear differentiation of the two species along the two first PCs (62.9% of the total variance). Differentiation mainly concerns three indices: the shape of the ambitus (AMB), the relative position of the apical system (RAS) and the posterior/ anterior ratio between petals (RPET). Congruent results of the two PCAs show that specimens of *A. bidens* have a more extended labrum, a more elongated ambitus, a more anterior apical system and a longer posterior petal than specimens of *A. cavernosus*. This morphological difference is also supported by the discriminant analyses performed on raw parameters and shape indices, with a high attribution ratio of 98.7% for both parameters and indices (Hotelling's T-squared: $p=9.3\ 10^{-15}$ and $p=2.3\ 10^{-19}$ respectively).

Morphological variations between the two genetic groups of *A. bidens*, G1 and G2, were also analyzed. No conspicuous difference was noticed between the two groups when specimens were observed at the naked eye and under the binocular microscope, but statistical analyses revealed significant differences for three following parameters: test height (HT), labrum extension (LB), and distance between the apical system and the beginning of petal II (DP) (Table 3). Variation ranges, however, widely overlap and clear, diagnostic morphological characters cannot be identified to differentiate the two groups. These results are also supported by the PCAs performed on raw parameters (Fig. 5) and shape indices independently. The PCA of raw parameters shows no clear differentiation between morphologies of the two groups along the first two PCs (Fig. 5a), the two groups best differentiating along PC3, which only accounts for 9.35% of the total variance, though widely overlapping with one another (Fig. 5b). These results are congruent with those of the discriminant analyses (Hotelling's T-squared: $p=1.6\ 10^{-5}$ and p=0.005 for raw parameters and shape indices respectively), the two groups best differentiating for raw parameters. This discriminant analysis performed on raw parameters also shows that the two groups of *A. bidens* partly overlap with one another in morphology but clearly differentiate from *A. cavernosus* (Fig. 6).

Morphological comparison of the three haplotypes demonstrates that they all differ significantly from each other in some of the measured, raw parameters. Pairwise Mahalanobis distances between the three groups were all tested significant regarding raw parameters, while for shape indices only the Mahalanobis distance between G1 and G2 is not significant (Table 4). Haplogroup G1 turns out to be the most different from G3 in morphology, while G2 is intermediate in position. This totally agrees with genetic results, the distance between G1 and G3 being the longest as well (Fig. 3a).

Besides morphometric analyses, qualitative characters were also examined to complement the morphological study, including observation of appendages (Table 5; Fig. 7). Some characters appear clearly diagnostic of *A. cavernosus* and allow to differentiate it from *A. bidens*. In contrast, no single character allows to differentiate the two groups of *A. bidens* from one another.

The test outline is heart-shaped in *A. cavernosus*, it is angular with a truncated posterior end in *A. bidens*. The width/length ratio is significantly different between the two species, *A. bidens* being the most elongated (unilateral t test on AMB: p=2.16 10^{-6}). All petals are almost of the same size in *A. bidens* (mean posterior petals size reaches 93% of that of anterior ones), while posterior petals are shorter in *A. cavernosus* (mean ratio = 64%). The color of test and spines is light brown in *A. bidens*, darker in *A. cavernosus*. The fasciolar band appears beige in *A. bidens*, in which it passes very low anteriorly, and dark purple in *A. cavernosus*. The apical system is anterior in position in *A. bidens*, centered in *A. cavernosus* (it is situated at 39% and 51% of total test length in *A. bidens* and *A. cavernosus* respectively).

Regarding plate patterns, in *A. cavernosus* the labrum is short and does not extend farther than the 1st, infrequently the 2nd adjacent ambulacral plate, while in *A. bidens* the labrum extends as far as the 3rd adjoining ambulacral plate, more rarely the 2nd and very seldom the 1st one. Marsupia abut the apical system in *A. cavernosus*, what is the most frequent pattern showed by Antarctic brooding schizasterids, while they often do not start before plates 8 or 9 in anterior petals of *A. bidens*, and plates 5 or 6 in the posterior ones (Fig. 8).

Pedicellariae of the two species were observed in details (Fig. 7). Globiferous pedicellariae are drastically distinct between the two species. They are not very numerous in *A. cavernosus*, appear black in living specimens when not cleaned of their soft tissue, and valves terminate in a series of four (rarely three) sharp, tiny teeth that are rooted on the upper margin of a circular opening (Fig. 7a). In *A. bidens*, globiferous pedicellariae are extremely numerous on the apical side, they are embedded in white tissue in living specimens, which turns dark in the specimens fixed in ethanol. Valves terminate in two to three very long hooks (Fig. 7d). Rostrate pedicellariae of the two species are relatively similar in shape except for the presence of a narrow, spiky distal tip in *A. bidens* (Fig. 7f), while it is blunt and serrated in *A. cavernosus* (Fig. 7b). Tridentate pedicellariae belong to two main types: (1) the classic type with a spoon-shaped distal part that tappers progressively toward the base and (2) a showel-shaped type with the distal part separated from the base by a more or less straight shaft (Fig. 7c, e). Both types are present in the two species, but they are more elongated in *A. cavernosus* (Fig. 7c) than in *A. bidens* (Fig. 7e).

Sphaeridae are somehow rounded in *A. cavernosus* (Fig. 7g), more elongated in *A. bidens* (Fig. 7h).

Systematics

The systematics and taxonomy of *A. bidens* is revised below based on the new genetic and morphological results obtained in the present work. All results are congruent and show that *A. bidens* should be regarded as a distinct

species, following David et al. (2005a), and not a variety (sub-species) of *A. cavernosus*, contrary to Mortensen (1951) and Kroh (2015). The description includes the two haplogroups of *A. bidens* identified in this study, G1 and G2, herein treated as two putative cryptic species. All the specimens figured and studied in the present work are housed in collections of the University of Burgundy, Dijon (France).

Abatus bidens **Mortensen 1910**

Figures 7 and 8

Abatus cavernosus var. *bidens* Mortensen, 1910; p. 73; Pl. 19: 32, 35, 39, 42.
Abatus cavernosus var. *bidens* Mortensen, 1951; p. 256.
Non *Abatus bidens* Bernasconi, 1953; p. 44; Pl. 24: 1–6; Pl. 25: 1–4.
Abatus bidens David et al., 2005a: 190–191.
Abatus cavernosus bidens Kroh, 2015:http://www.marinespecies.org/ophiuroidea/aphia.php?p=taxdetails&id=513701
Abatus bidens Kroh, 2015: http://www.marinespecies.org/ophiuroidea/aphia.php?p=taxdetails&id=160761

Material: see Table 1 for location of material examined.

Diagnosis (Table 5): apical system anterior in position. Anterior and posterior petals of equal length. Anterior branch of the fasciole low on the test. Labrum extending posteriorly to the 2nd or 3rd adjacent ambulacral plate. Globiferous pedicellariae very numerous on the apical side, particularly between petals and on the two margins of the anterior ambulacrum, with valves terminating in two or three long hooks positioned at the end of a narrow, elongated aperture (Fig. 7d). Living specimens yellow with whitish globiferous pedicellariae.

Description

General morphology and plate pattern.

Size: The mean length of adult specimens is 46.7 mm, the females being slightly larger than males (unilateral t test: p= 0.008).

Color: Living specimens beige to light brown, sometimes almost yellow (Fig. 8). The color of spines is light beige. The fasciole is of the same color, turning dark in dry specimens. Plate pattern of the apical side is more or less shown by subtle changes in coloration.

Outline of the test: Ambitus anteriorly rounded with a faint frontal notch and somehow posteriorly truncated, making an angle covered with a tuft of spines on each side. Test width is 89% to 98% of test length (94% on average). The posterior end of the test is vertically truncated, otherwise dome-shaped. Test height is 60% of test length. Posterior petals are sligthly shorter than anterior ones and converge at a more acute angle than anterior ones.

Apical system: It is slightly anterior in position, at 39% of the total test length from the anterior. Plating follows the classic *Abatus* ethmolytic pattern with three gonopores (there is no gonopore in genital plate 2). Gonopores larger in females.

Periproct: Located on the vertical posterior side, its adoral margin situated at 42% of test height from the lower surface. It is scarcely visible in either apical or oral views. It is embedded between interambulacral plates 5.a.4/5.b.5 or 5.a.5/5.b.6 adorally, and 5.a.7/5.b.7 or 5.a.7/5.b.8 apically.

Peristome: It is relatively large and discernible as the anterior part of the labrum does not overhang over it. It is anterior in position, located at 31% of test length from the anterior of the test.

Plastron: In interambulacrum 5, the labrum is long, extending backward to the 3rd or 2nd adjacent ambulacral plates (sometimes the 4th).

Marsupiae (petals) and sexual dimorphism: Anterior and posterior petals almost of the same size, especially in haplogroup G1 (mean ratio = 0.97) compared to haplogroup G2 (mean ratio = 0.87). In most specimens, the adapical extremity of brood pouches is distant from the apical system, a morphological feature diagnostic of *A. bidens*. It is particularly conspicuous in females, in which anterior pouches initiate as far as the 8th to 9th ambulacral plates, while posterior ones are less distant from the apical system. This character is reminiscent of the plate pattern observed in *Abatus nimrodi*.

Fasciole: The peripetalous orthofasciole (*sensu* Néraudeau et al. 1998) is very conspicuous and form a broad band of 7 to over 15 rows of miliaries, depending on the segment considered and on test size. The fasciole is excentric anteriorly with the posterior segment distant from the posterior end of the test, and the anterior segment passing very close to the ambitus, barely visible in apical view.

Appendages

Primary spines: They are relatively coarse and of the usual schizasterid shape with finely serrated longitudinal ridges. The largest spines occur on the adoral side, in the plastronal and other interambulacral areas, and are used for locomotion. Their distal extremity is gently curved. The smallest spines are on the apical side and show rounded tips.

Secondary spines: Those observed on specimens collected at station 163-9 (Weddell Sea) are club-shaped and densely distributed.

Miliaries: They are made of a relatively loose stereom meshwork composed of 7 to 8 longitudinal ridges separated by large openings. They end in a swollen serrated tip.

Clavulae: They are straight, made of fenestrated stereom, and end in a tuft of trabeculae. They are very similar in *A. bidens* and *A. cavernosus*.

Pedicellariae: The globiferous pedicellariae are extremely numerous, particularly on the apical side in the vicinity of petals and along each side of ambulacrum III. In living specimens, they appear as vivid yellow or whitish spots; they are brownish in specimens preserved in ethanol. Their valves terminate in two or three long hooks located at the top of an elongated opening (Fig. 7d). This character is shared with several other species of *Abatus* (e.g. *A. curvidens*, *A. agassizi*, and *A. elongatus)* and can putatively be regarded as plesiomorphic as it is also present in several species of *Amphipneustes* and *Tripylus*.

Tridentate pedicellariae (dentate pedicellariae are very commonly tridentate, sometimes bidentate) belong to two main types. As indicated above, valves of type 1 have a spoon-shaped distal part that tappers progressively toward the base (Fig. 7e). Valves of type 2 comprise three parts: the base, the intermediary tubular part, and the spoon-shaped, and finely serrated distal part (Fig. 7e). Rostrate pedicellariae display a very long and curved blade with small lateral teeth along the rim of the blade (Fig. 7f).

Sphaeridiae: They are slightly ovoid and ornamented with smal meridian ridges and grooves (Fig. 7h).

Distribution: *A. bidens* was reported with confidence by the Swedish South Polar and Discovery expeditions from the South Georgia Islands, between 64 and 270 m (Mortensen 1910), and along the Antarctic Peninsula in the Bransfield Strait and in the northwest of the Weddell Sea, between 102 and 782 m (this study).

Remarks: Mortensen (1951) regarded *A. bidens* as a subspecies of *A. cavernosus* but suggested that it could be a distinct species. David et al. (2005a) considered *A. bidens* as a separate species because globiferous pedicellariae of the two species are very different. Following Mortensen (1951) and David et al. (2005a), the specimens described by Bernasconi (1953) as *A. bidens* are considered here as representatives of *Tripylus excavatus* because adult individuals possess a conspicuous latero-anal fasciolar branch, absent in *A. bidens*. The two cryptic species (genetic groups G1 and G2) are here referred to as *A. bidens* pending for new data.

Discussion

In the present study, morphological and genetic results show that the two nominal species *A. cavernosus* and *A. bidens* are clearly distinct and should be regarded as truly different species as formerly suggested by David et al. (2005a). Several morphological features were recognized as clear, diagnostic characters of *A. bidens*, among which the number and shape of globiferous pedicellariae are the most remarkable (Table 5). Other distinctive, and more accessible characters, albeit not so clear cut, are petal relative size (anterior and posterior petals are of the same size in *A. bidens*) and the position of the apical system (anterior in *A. bidens*). These characters are reliable enough for the field determination of the species.

Unexpectedly, two genetic groups of *A. bidens* were identified. The genetic divergence between the two groups (2.7%) is significant enough to suggest that they could be reproductively isolated from each other. This is congruent with results obtained with molecule COI for other echinoderms. Genetic distance between closely related species of echinoderms usually ranges between 0.62% and 1.3% (Ward et al. 2008; Hoareau and Boissin 2010). Some cryptic species can even display up to 3% of genetic divergence (Egea et al. 2015). For instance, the most closely related lineages within the species complex *Ophioderma longicauda* display a COI divergence of 2.2% (Boissin et al. 2011), and cryptic species of the sand dollar *Mellita longifissa* show a genetic divergence comprised between 5.60% and 3.97% (Coppard et al. 2013). Hence, in the present study, the 2.7% of divergence between haplogroups G1 and G2 of *A. bidens*, the absence of intermediate forms between them, and the short genetic distance between them and *A. cavernosus* (3.5% to 5%) strongly suggest that they constitute two cryptic species, all the more as schizasterid echinoids were shown to present slow rates of mitochondrial DNA evolution (Chenuil et al. 2008, 2010). The lack of clear-cut discrimination within morphological variations of the two forms of *A. bidens* is in line with this interpretation. Some morphological differences between the two genetic groups were tested significant but most variations significantly overlap. Three evolutionary scenarios, however, can challenge our interpretation because they too can generate divergent lineages in a biological species with no intermediate forms: a past demographic bottleneck, a selective sweep, and a long-lasting vicariance that did not result in an efficient reproductive barrier before "secondary" contact. The only way to rule these scenarios out and prove that haplogroups G1 and G2 form true cryptic species of *Abatus bidens*, experimental crosses excepted, would consist in characterizing at least one additional genetic marker independent from the mitochondrial COI gene that is, a DNA nuclear marker.

The two groups of *A. bidens* were collected in sympatry at the scale of sampling stations (they were found together at five of the eight stations at which they were identified). *A. cavernosus* was found living in sympatry with

A. bidens at five stations of the 12 stations at which it was collected, and the three haplogroups were found together at four of these stations. Despite the limited number of sampling sites, the occurrence of species together was frequent and attests that they can be living in sympatry. The co-existence of closely related species of similar forms is generally considered a rare phenomenon as sympatry presumes the existence of differences in ecological niches and life history traits. In environments of the Southern Ocean, such cases are known in amphipods (Baird et al. 2011), isopods (Held 2001), and crinoids (Hemery et al. 2012). In irregular echinoids, sympatry was observed between closely related species of the genus *Echinocardium* (between *Echinocardium cordatum* and *Echinocardium flavescens* in Norway, pers. obs.), as well as between species of the sand dollar *Mellita* along the tropical coasts of the Americas (Coppard et al. 2013). Sympatry between attested, cryptic species is much more rare. Examples are documented in the sand dollar *Mellita*, in which only one of nine possible cases of sympatry between cryptic species was recorded (Coppard et al. 2013), and in the species complex *E. cordatum* (Egea et al. 2011; Egea et al. 2015). Contrary to these two case studies, all species of *Abatus* are brooders and consequently, show limited dispersal capability compared to sea urchins with planktonic larvae. The co-existence of *A. bidens* and *A. cavernosus* at the same sites (Fig. 3c) is therefore unexpected, all the more that previous studies revealed significant genetic differentiation between populations of *Abatus* species at small spatial scale such as in *Abatus cordatus* (Poulin and Féral 1995; Ledoux et al. 2012), *Abatus agassizi* (Díaz et al. 2012), and *Abatus nimrodi* (Chenuil et al. 2004). In the present case study, the mechanisms involved in the separation between the three forms cannot be uncovered with confidence. Morphological similarities exclude the hypothesis of a strict ecological mechanism at the origin of the divergence for the three groups, and the two forms of *A. bidens* in particular probably have similar modes of life. The separation is more likely rooted in life history traits (e.g. shifts in spawning seasons) or in prezygotic isolation (i.e. incompatibility between gamete recognition proteins) (Lessios 2011). Finally, it cannot be excluded that haplogroups of *A. bidens* may interbreed with no fitness cost and belong to the same biological species, nor that post-zygotic incompatibility only is involved in the isolation of the two forms despite the evolutionary cost it would imply.

At the scale of the three geographic areas explored, the two forms of *A. bidens* were not collected in the Drake Passage, while conversely, *A. cavernosus* was frequently found there (at 83% of the sampling sites). The latter species is also present in the Bransfield Strait but it was less frequently found than *A. bidens*, and it is absent from the northwest of the Weddell Sea (namely no sequence of *A. cavernosus* was identified in the Weddell Sea) (Fig. 9). Two distinct distribution patterns seem to appear and to follow reverse, latitudinal gradients: *A. cavernosus* is the most frequently encountered to the north, and most often outside the areas strongly impacted by sea ice, while the two forms of *A. bidens* are the most frequent to the south in areas under sea ice influence (Fig. 9). Though still in need for further research, these apparent patterns are supported by records of *A. cavernosus* at the tip of South America (Bernasconi 1925, 1966) and by the putative presence of *A. bidens* in Adelie Land (Koehler 1926) described globiferous pedicellariae of "*A. cavernosus*" with two long hooks, suggesting that its specimens should be regarded as *A. bidens*). The two species have been also unquestionably recognized to co-occur in South Georgia (Bernasconi 1953). This is not contradictory with the proposed pattern as South Georgia has been often regarded as a biogeographic area under the cross influence of Antarctic and sub-Antarctic regions. Although unevenly distributed, the three haplogroups co-occur in the Bransfield Strait where they can be found in sympatry. Such a diversity pattern has already been highlighted in previous studies for other organisms (Wilson et al. 2007; Hemery et al. 2012). Along with the Scotia Arc region and the northern tip of the Antarctic Peninsula, the Bransfield Strait is a favored zone of

connectivity among populations due to the strong current systems present near the tip of the Antarctic Peninsula (Thompson et al. 2009).

At the scale of the entire Southern Ocean, the attested occurrence of *A.bidens* in the Peninsula area as well as in Adelie Land suggests that the species has a wide, potentially circumpolar distribution. The respective distribution of the two halpogroups still remain to be clarified, in East Antarctica in particular. In contrast, *A. cavernosus* seems to have a more northern distribution, potentially restricted to the Magellanic and Scotia Arc regions as no report of *A. cavernosus* outside this area is fully certain. This distribution pattern is in complete opposition with previous works (David et al. 2005a).

Conclusion

Pending for further genetic analyses including closely related species of *Abatus* (i.e. *Abatus philippi*, *A. agassizi*, and *A. cordatus*) and additional samplings in other areas of the Southern Ocean, the present study, however, suggests that the *A. cavernosus* - *A. bidens* complex comprises at least two morphologically close species and two cryptic species. It contributes to clarifying the systematics of *A. cavernosus* and *A. bidens* and shows that *A. bidens* should definitely be regarded as distinct from *A. cavernosus*, based on congruent morphological characters and genetic results. In addition, *A. cavernosus* and the two forms of *A. bidens* were shown to have distinct distribution patterns.

The present work is also the first study to report the occurrence of cryptic species of echinoids in the Southern Ocean, in accordance with many observations of various marine invertebrates of the Southern Ocean. It leads to revisit the richness of the Antarctic echinoid fauna, its systematics, macroecological patterns, and to re-assess the underpinning evolutionary processes and history. It also highlights the crucial importance of associated morphological and genetic studies for improving our knowledge of Antarctic biodiversity. This is a prerequisite for further process-based studies, from ecology to phylogeography, and for uncovering the origin of the rich Antarctic biodiversity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure and table captions

Fig. 1 The 19 stations in the Weddell Sea (yellow dots), Bransfield Strait (orange dots), and Drake Passage (blue dots) where the studied specimens of *Abatus cavernosus* and *Abatus bidens* were collected during cruise PS81 of the R/V *Polarstern*

Fig. 2 Measurements performed in the morphometric analysis. a, echinoid test drawn in apical view (fasciolar band in light grey, brood pouches in black, apical system detailed in the center). b, test in posterior view (fasciolar band in light grey, brood pouches and periproct in black). c, test in oral view with details of plate patterns (peristome in black). a, total test length (LL) and width (WD), distance between the apical system and the back of the test (AS), length of posterior petal I (PPT), length of anterior petal II (APT), distance between center of the apical system and beginning of petal (pouch) II (DP). b, total test height (HT), distance between the periproct and the base of the test (PP). c, distance between the peristome and the back of the test (PS); total number of adjacent ambulacral plates abuting the labrum (LB). A maximum of six ambulacral plates (3 per ambulacrum) can abut the labrum in *Abatus bidens*; two ambulacral plates only abut the labrum in the specimen drawn in Figure 2c

Fig. 3 Median-joining network of haplotypes based on mtDNA COI sequences obtained from 5 specimens of *Abatus cavernosus* and 44 specimens of *Abatus bidens*. a, each haplotype is labelled from H1 to H8, and shown in a colored circle indicating the genetic unit to which it belongs: G1 (light green), G2 (dark green), and G3 (red). Size of circles proportional to frequency in the sample analyzed. Mutations shown with unlabelled red dots. Minimum pairwise distances (minimum number of mutations) between specimens of the three groups shown in the table inserted. b, colors indicating the sampling geographic area of each haplotype: the Bransfield Strait (orange), Weddell Sea (yellow), and Drake Passage (blue). c, colors indicating the sampling stations of each haplotype

Fig. 4 Principal component analysis of the ten raw morphometric parameters measured in specimens of *Abatus bidens* (green dots) and *Abatus cavernosus* (red dots). The two species differentiate along the first two principal components, which account for 75.06% of the total variance

Fig. 5 Principal component analysis of the ten raw morphometric parameters measured in specimens of *Abatus bidens* (G1, light green dots; G2, dark green dots). a, the first two principal components, which acount for 78.82% of the total variance. b, first and third principal components, which accounts for 76.53% of the total variance

Fig. 6 Discriminant analysis of the ten raw morphometric parameters measured in specimens of *Abatus bidens* (G1, light green dots; G2, dark green dots) and *Abatus cavernosus* (G3, red dots)

Fig. 7 Pedicellariae and sphaeridiae in *Abatus cavernosus* (a-c, g) and *Abatus bidens* (d-f, h). a, isolated valve of globiferous pedicellaria from specimen UBGD 279061 (G3). b, valves of rostrate pedicellariae from specimens UBGD 279063 (G3), UBGD 279061 (G3), and UBGD 279064 (G3) from left to right. c, valves of tridentate pedicellariae from specimens UBGD 279062 (G3), UBGD 279064 (G3), and UBGD 279063 (G3) from left to right. d, valves of globiferous pedicellariae from specimens UBGD 279067 (G1) and UBGD 279070. e, tridentate pedicellariae from specimen UBGD 279067 (G1). f, rostrate pedicellariae from specimens UBGD 279069 (G1), and UBGD 279070. g, sphaeridiae from specimens UBGD 279062 (G3) and UBGD 279064 (G3). h, sphaeridiae from specimens UBGD 279070 and UBGD 279068

Fig. 8 *Abatus cavernosus* (a-d) and *Abatus bidens* (e-h). a, apical view of fresh specimen UBGD 279059. b, apical, c, oral, and d, posterior views of denuded test of specimen UBGD 279060. e, apical view of fresh specimen UBGD 279065. f, apical, g, oral, and h, posterior views of denuded test of specimen UBGD 279066. Scale bars: 10 mm

Fig. 9 Distribution map of the genetic groups of *Abatus cavernosus* and *Abatus bidens* (G1, light green; G2, dark green; G3, red) among the 14 stations where they were identified. Circle wedges indicate presence/absence of genetic groups, not abundance

Table 1 Sites where specimens of the species complex *Abatus cavernosus – Abatus bidens* were collected. Crosses indicate where the haplogroups were formally identified and where non-sequenced specimens were identified based on morphology alone.

Sampling sites	*Abatus bidens*			*Abatus cavernosus*	
	haplogroup G1	haplogroup G2	non-sequenced	haplogroup G3	non-sequenced
Weddell (162-7)		X			
Weddell (163-9)	X	X			X
Weddell (164-4)	X	X			X
Weddell (188-4)			X		
Weddell (190-2)			X		
Bransfield (116-9)				X	
Bransfield (196-8)			X		
Bransfield (197-5)	X				
Bransfield (198-5)		X			X
Bransfield (199-4)			X		
Bransfield (204-2)	X	X			
Bransfield (205-1)			X		
Bransfield (220-2)	X	X		X	
Bransfield (224-3)	X	X		X	
Bransfield (227-2)	X				
Drake (234-5)				X	
Drake (237-3)					X
Drake (240-3)					X
Drake (245-1)					X

Table 2 Morphological differences between haplogroups of *Abatus bidens* and *Abatus cavernosus*. The table gives the probability values for Student bilateral tests. Significant values only are detailed (abbreviations are given in Fig. 2)

Parameters	LL, WD, HT, PP, APT	PS	AS	PPT	DP	LB
p values	> 0.05	0.004	$1.6\ 10^{-7}$	$1.2\ 10^{-9}$	0.015	$1.3\ 10^{-6}$

Table 3 Morphological differences between haplogroups G1 and G2 of *Abatus bidens*. The table gives the probability values for Student bilateral tests. Significant values only are detailed (abbreviations are given in Fig. 2)

Parameters	LL, WD, PS, PP, AS, PPT, APT	HT	DP	LB
p values	> 0.05	0.02	0.01	0.01

Table 4 Pairwise Mahalanobis distances between haplogroups G1, G2, and G3 of *Abatus bidens* and *Abatus cavernosus* respectively. The table gives the probability values for comparisons using raw variables (matrix upper half) and shape indices (matrix lower half). Asterisks indicate if differences are significant (*) or highly significant (**)

	G1	G2	G3
G1		0.0368*	0.0005**
G2	0.7518		0.0292*
G3	0.0007**	0.0064**	

Table 5 Diagnostic morphological characters of *Abatus bidens* (including the two genetic groups) that clearly differ from those of *Abatus cavernosus*. Characters are listed in order of importance for distinguishing the two species

	A. bidens	*A. cavernosus*
Globiferous pedicellariae	They are numerous, particularly on the apical side. Their valves terminate by two long hooks located above a narrow, elongated aperture.	They are rare. When present, a series of three to four teeth borders the upper rim of a rounded aperture.
Petals	Posterior petals about the same length as the anterior ones.	Posterior petals shorter than the anterior ones.
Color	Living specimens yellow. Globiferous pedicellaria whitish.	Living specimens brownish.
Apical system and fasciole	Shifted anteriorly.	Centered.
Labrum	It extends posteriorly to the 2nd or 3rd adjacent ambulacral plates.	It extends posteriorly to the 1st (or 2nd) adjacent ambulacral plates.
Brooding pouches (in females)	They often start away from the apical system.	Always starting close to the apical system.
Sphaeridiae	Elongated	Rounded or flattened

Figure 1

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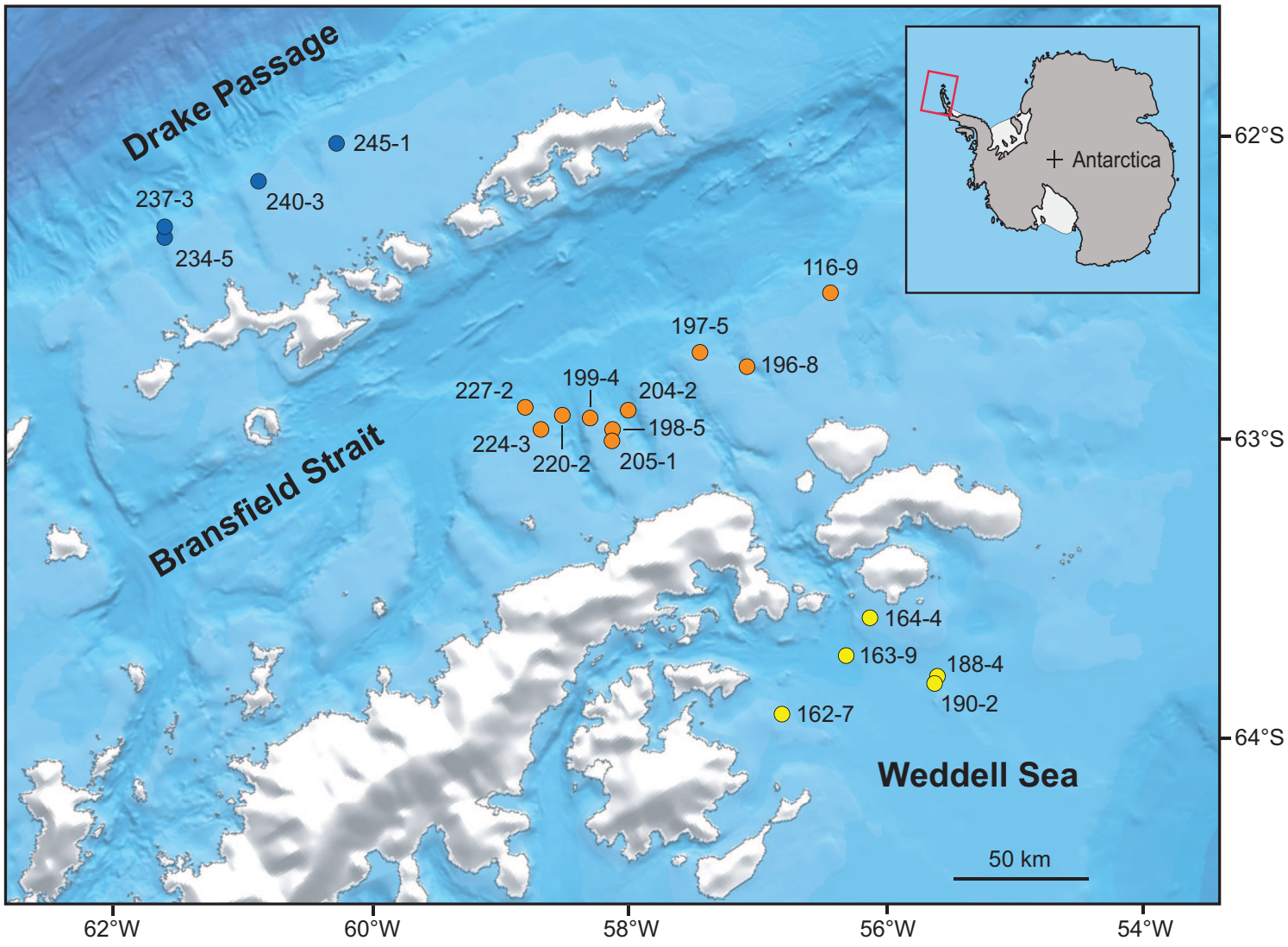

Figure 2

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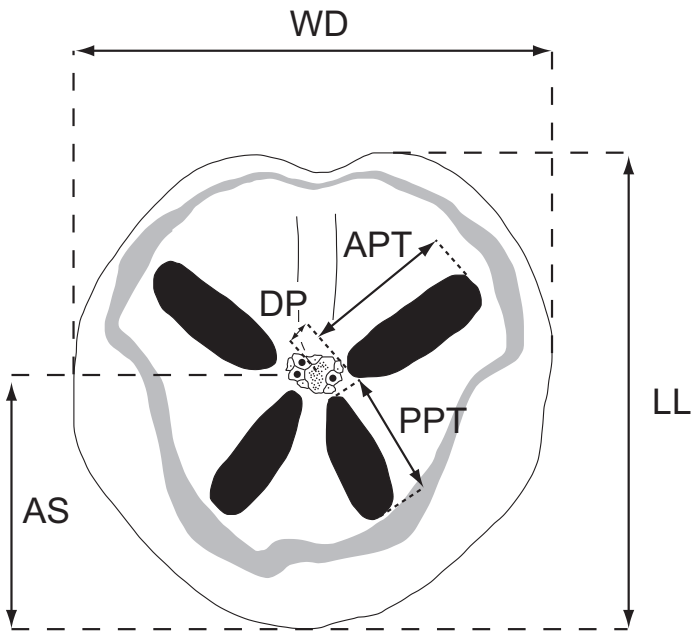
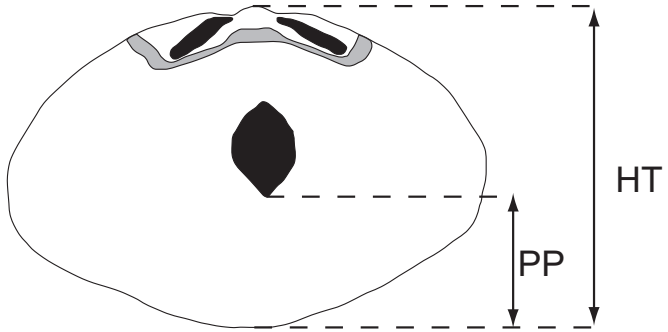


a



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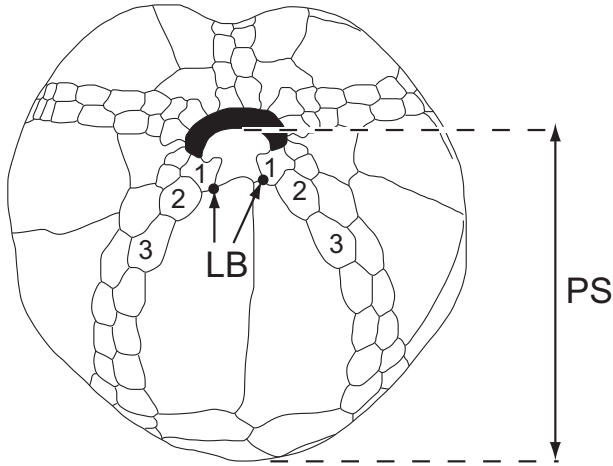

c

Figure 3

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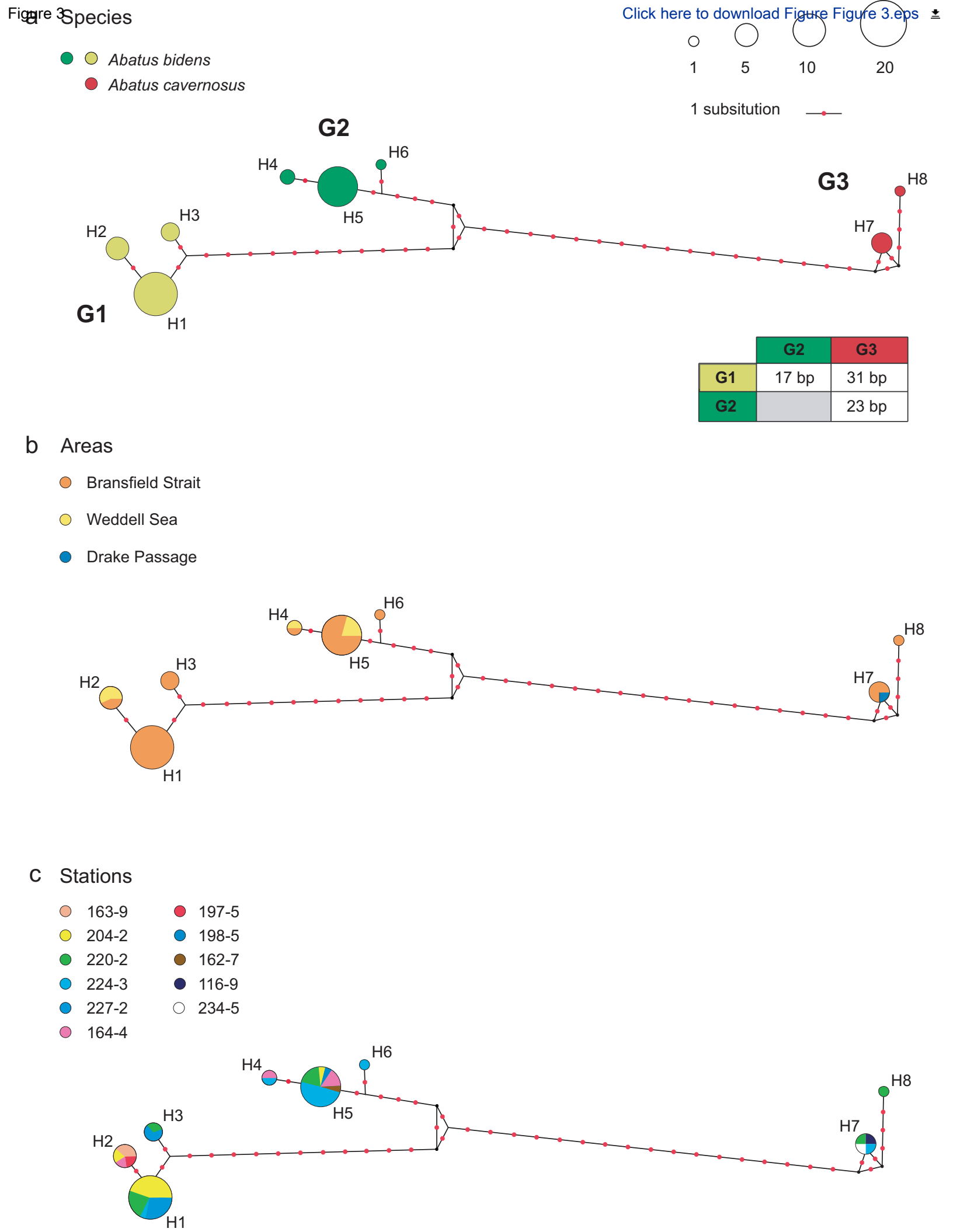

Figure 4

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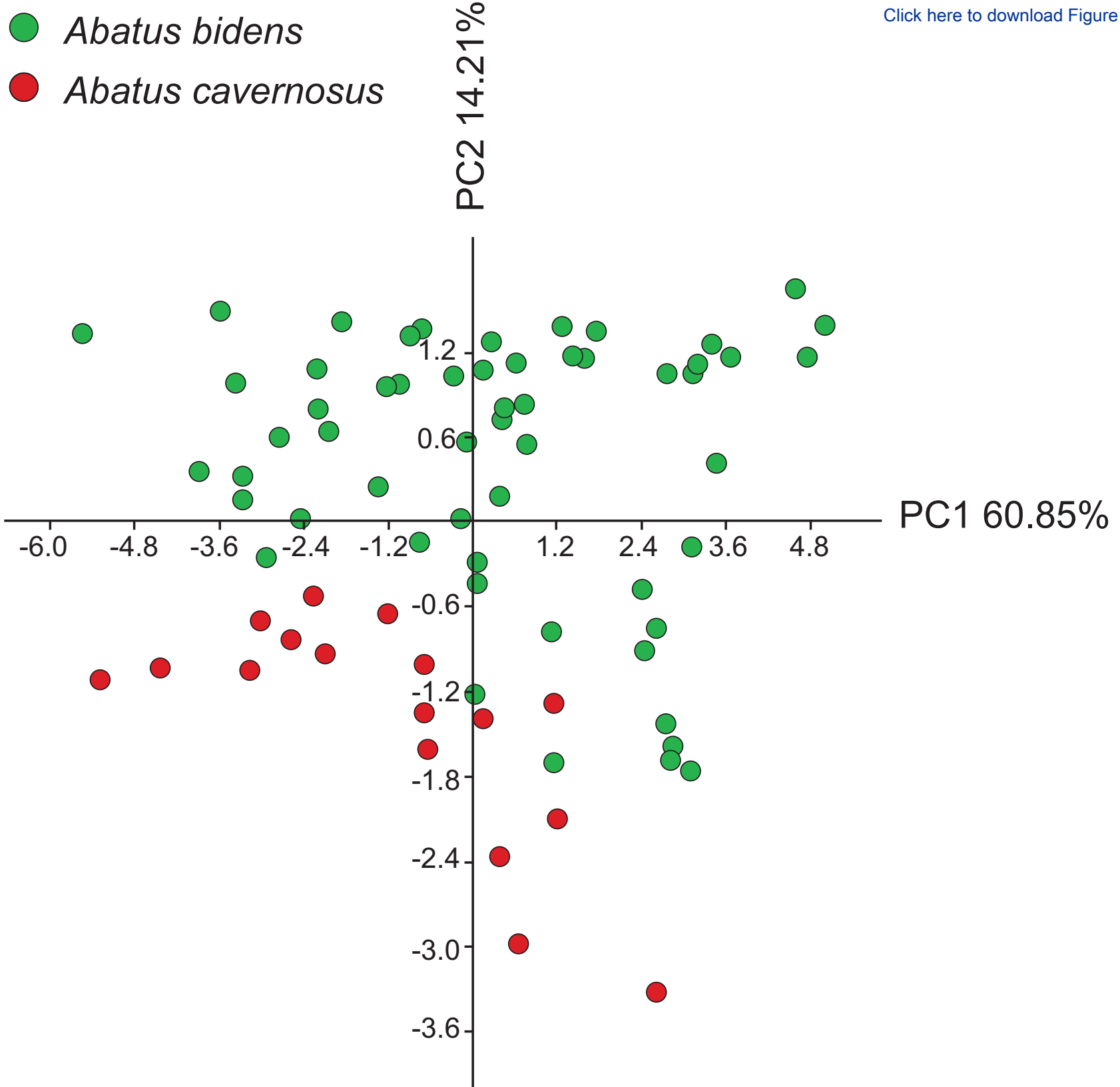

Figure 5

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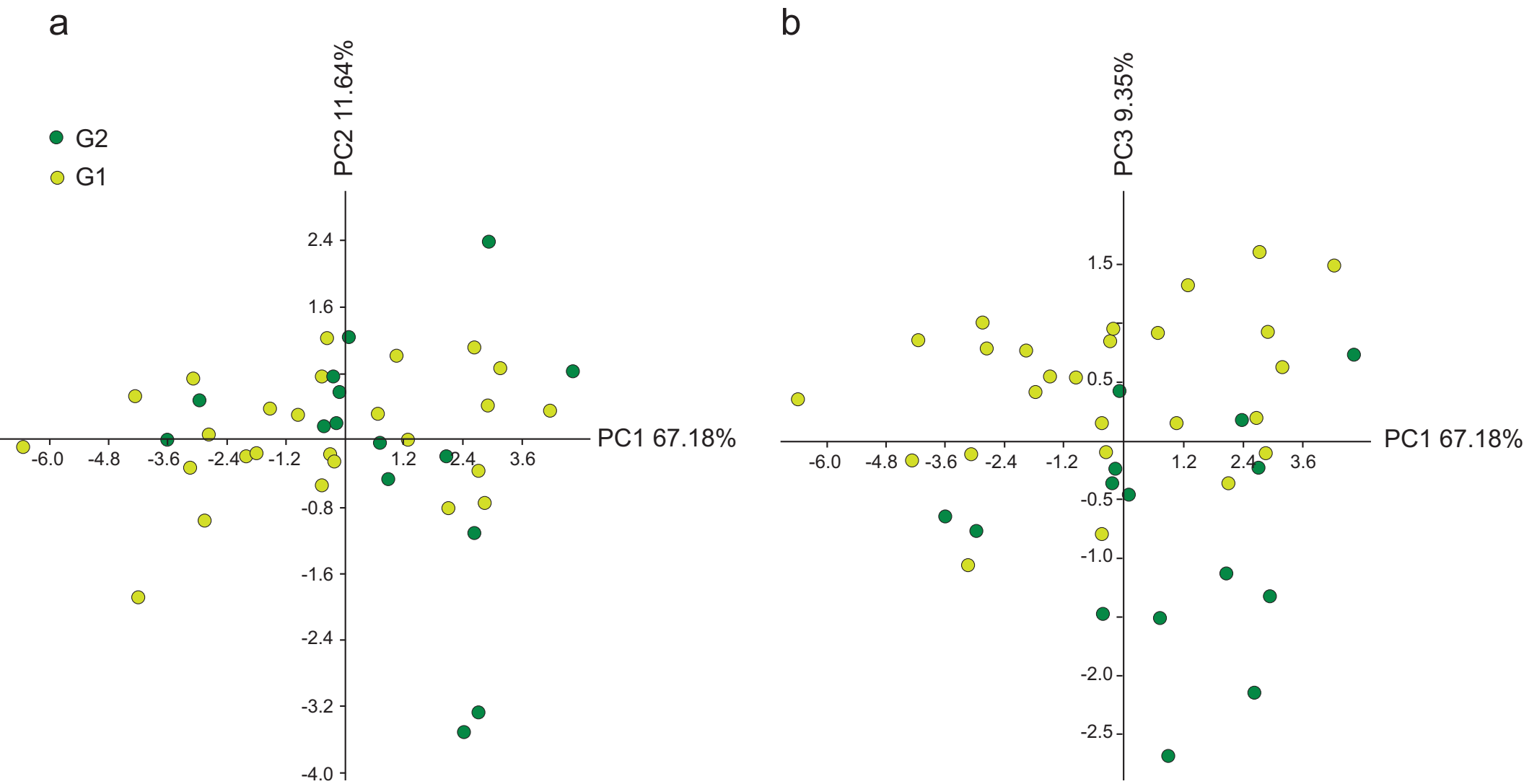

Figure 6

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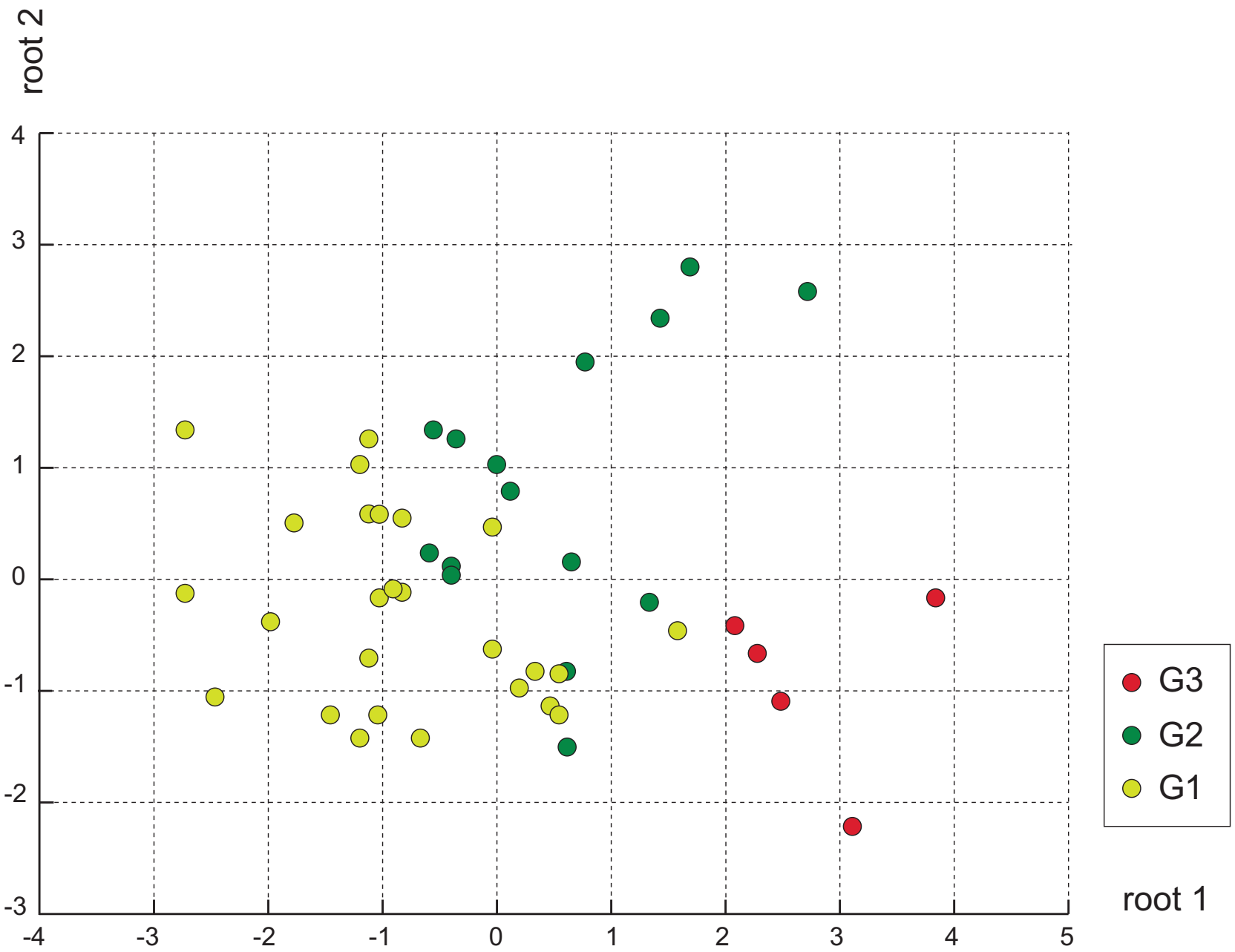

Figure 7

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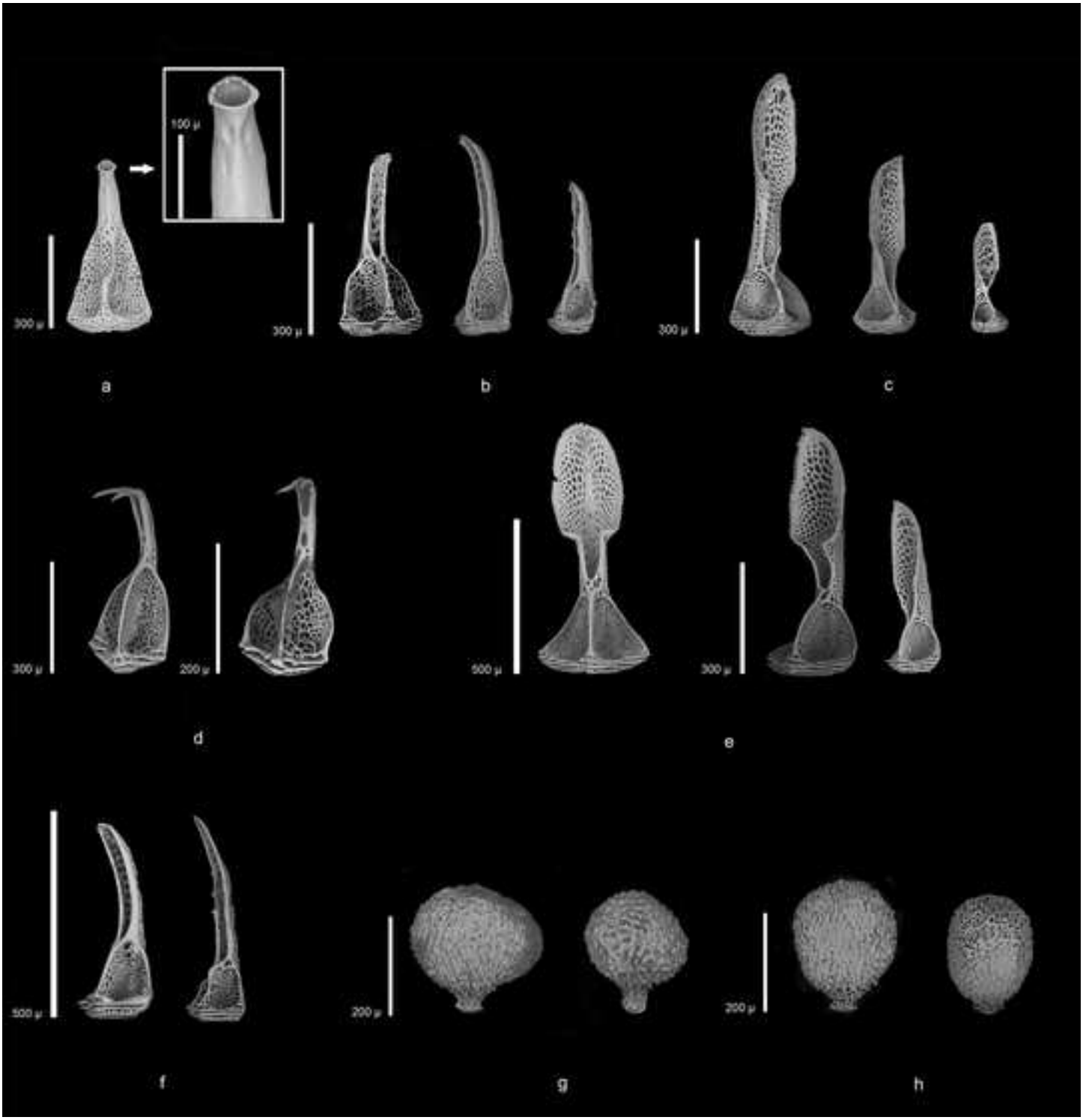

Figure 8

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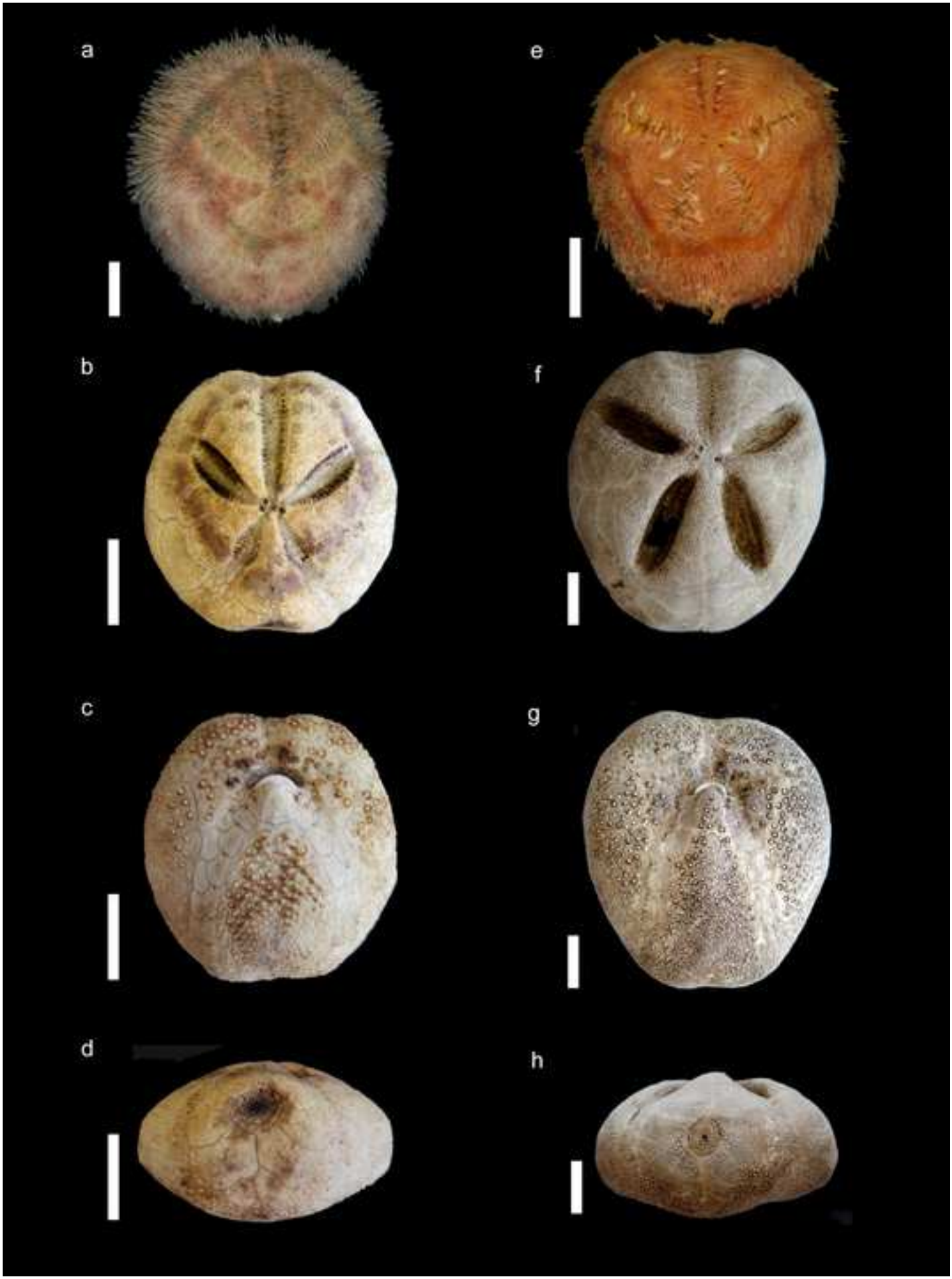

Figure 9

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