Spiny fruits revealed by nano-CT scanning: *Pseudoanacardium peruvianum* (Berry) gen. et comb. nov. from the early Oligocene Belén flora of Peru

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ABSTRACT. Fossil fruits formerly described as cashews from the Oligocene of Peru are reinvestigated based on the original specimens and newly collected materials. The recovery of an outer spiny layer, preserved in the sedimentary molds surrounding the locule casts, indicates that these disseminules do not represent *Anacardium*. Imagery from nano-CT scans of the specimens documents a distinctive morphology which does not resemble any fruits or seeds of Anacardiaceae. We describe the morphology in more detail and reassign the fossils to an extinct genus, *Pseudoanacardium* gen. nov., of uncertain familial affinity. *Pseudoanacardium peruvianum* (Berry) comb. nov. was a prominent member of the Belén carpoflora, which also included palms plus Annonaceae, Euphorbiaceae, Humiriaceae, Leeaceae, Icacinaceae, Rutaceae and Vitaceae.

KEYWORDS: fruit, carpology, Anacardiaceae, paleobotany, Oligocene, Peru

INTRODUCTION

The Oligocene Belén flora of northwestern Peru contains a diverse assemblage of angiosperm fruits and seeds (Berry 1927, 1929, Manchester et al. 2012a). Families that have been confirmed based on fruit morphology include Annonaceae, Arecaceae, Euphorbiaceae, Humiriaceae, Leeaceae, Icacinaceae, Rutaceae and Vitaceae. Detailed investigations of the Humiriaceae (Herrera et al. 2010, 2014), Icacinaceae (Stull et al. 2012), Leeaceae and Vitaceae (Manchester et al. 2012b) have been published in recent years, and a reinvestigation of the entire assemblage is underway.

One of the most common and conspicuous fossils at the Belén locality is a cashew-shaped seed or fruit originally attributed to the extant genus *Anacardium* by Berry (1924, 1927, 1929). The general form and size of the specimens are similar to that of a modern cashew, but the assignment to *Anacardium* has been questioned (Manchester et al. 2007, 2012a). The original diagnosis was based on smooth internal casts lacking external surface features of the fruits. More recently recovered specimens from the type locality reveal spiny external ornamentation that Berry would not have been able to observe in his original study. These spiny fruits or seeds are unlike any known in the Anacardiaceae today, and it is clear that they do not belong to *Anacardium*.

The specimens are not fully permineralized, but instead are preserved as sedimentary molds and silica casts. The molds, preserving the original spiny external morphology, occur in the sediment that surrounds the more resistant silica casts. The intervening space between

the fruit molds and the silica locule casts replicates the original thickness of the fruit wall. The spiny surface was inferred earlier from the obvious external protrusions into the sediment (Fig. 5b in Manchester et al. 2012a), but the surface morphology has been difficult to document by traditional methods. We found that X-ray imagery, particularly nano-CT scan data, provided helpful visualization of the original morphology. Here we emend the diagnosis of Berry's species to include the new observations, and transfer the species to a new genus, *Pseudoanacardium*: *Pseudoanacardium peruvianum* (Berry) comb. nov. Possible affinities are discussed but the precise relationships among extant angiosperms remain unclear.

MATERIAL AND METHODS

We studied specimens from the original collections of Berry (1924, 1927) in the Natural History Museum collections of the Smithsonian Institution, Washington, D.C. (USNM), and additional collections made in 2010 by Fabiany Herrera, Steven Manchester and Francisco Navarro (Manchester et al. 2012a) housed at the Florida Museum of Natural History, University of Florida, Gainesville (UF). Specimens were photographed by reflected light using a Canon Rebel EOS XSi digital camera with a Canon EFS 60 mm macro lens.

Integrating CT scanning results with standard reflected light imagery yields an improved understanding of the original morphology of these spiny fruits. The scanning was performed initially on one specimen at the Shared Materials Instrumentation Facility, Duke University, using a Nikon XTH 225 ST micro-X-ray CT Scanner at 145 kV, with 1800 projections over 360°. Subsequently, several specimens were scanned at the University of Florida College of Engineering Nanoscale Research Facility (NRF) with a GE Phoenix V|tome|xm240 CT Scanner, using a Tungsten reflection target and 0.5 mm copper filter, at 210 kV and 270 µA, with 1900 images of a single specimen for voxel size of 65 µm.

Datasets from nano-CT were analyzed with Avizo 9.0 Lite (FEI Visualization Science Group, Bordeaux, France) to provide volume renderings, isosurface renderings, and virtual sections in transverse and longitudinal orientations. The original CT scan datasets are archived at FLMNH and selected exemplary datasets were uploaded to https://www.morphosource.org. Three-dimensional images were generated from the stack of imported two dimensional Tiff images and displayed via the volume-rendering and isosurface modules of Avizo. Virtual slices were prepared in various orientations using the "slice" module. Internal surfaces were generated according to the following protocol using the Avizo software. Isosurface mode was selected and the threshold adjusted to optimize details of the surface spines. Under the "more options" menu of the isosurface properties box, we then selected "create surface." The resulting "surf" file was highlighted and then "surface editor" was activated. After pausing for the data to load, the resulting image became visible on the screen when "draw style" in the surface properties window was changed from "outline" to "shaded." To achieve optimal 3-D effects it was necessary to enable shadows in the "view" menu of the main window and to activate them by clicking on the shadow icon (upper right of the surface view properties window). The results were saved by highlighting the newest green icon, choosing "Extract Surface" and pressing "apply" in the corresponding properties box. The resulting new icon that appeared in the Project View window ("Extracted Surface") was activitated; then under File Menu, we chose "export data as" and selected the PLY format. Snapshots and movies (Supplemental Materials) of the resulting surfaces were produced following methods described in the Avizo documentation. To experiment with additional surface rendering modes with varied shadowing effects, the PLY file was imported as a mesh into the open source program, Meshlab. In Meshlab, we found the filter option "remove isolated pieces (wrt diameter)" to be most useful for eliminating loose debris.

SYSTEMATIC PALAEOBOTANY

Family: Indet.

Genus: *Pseudoanacardium* gen. nov.

Type species. *Pseudoanacardium peruvianum* (Berry) comb. nov.

Generic Diagnosis. Fruits reniform, bisymmetrical, with keel in plane of symmetry, lensoidal to nearly circular in cross section, unilocular and single-seeded. Fruit wall ornamented with closely spaced spines on all surfaces except along elliptical ridge over ventral concavity. Spines terete, 1.6 mm long. Fruit wall from which the spines arise, ca 1 mm thick, represented only by space between silica locule cast and sedimentary impression of fruit. Locule cast convex dorsally, concave ventrally, rounded over one end, narrower and pointed at other end, with large circular scar ca 1/3 of full length of locule, positioned on central portion of ventral surface. Locule cast smooth or sometimes striate, with striae running oblique to plane of symmetry. Seed coat thin, shrunken away from locule.

Pseudoanacardium peruvianum (Berry) comb. nov.

Pl. 1, 2

Basionym. *Anacardium peruvianum* Berry 1924. Am. J. Sci., vol. 8, p. 124, figs 1–7.
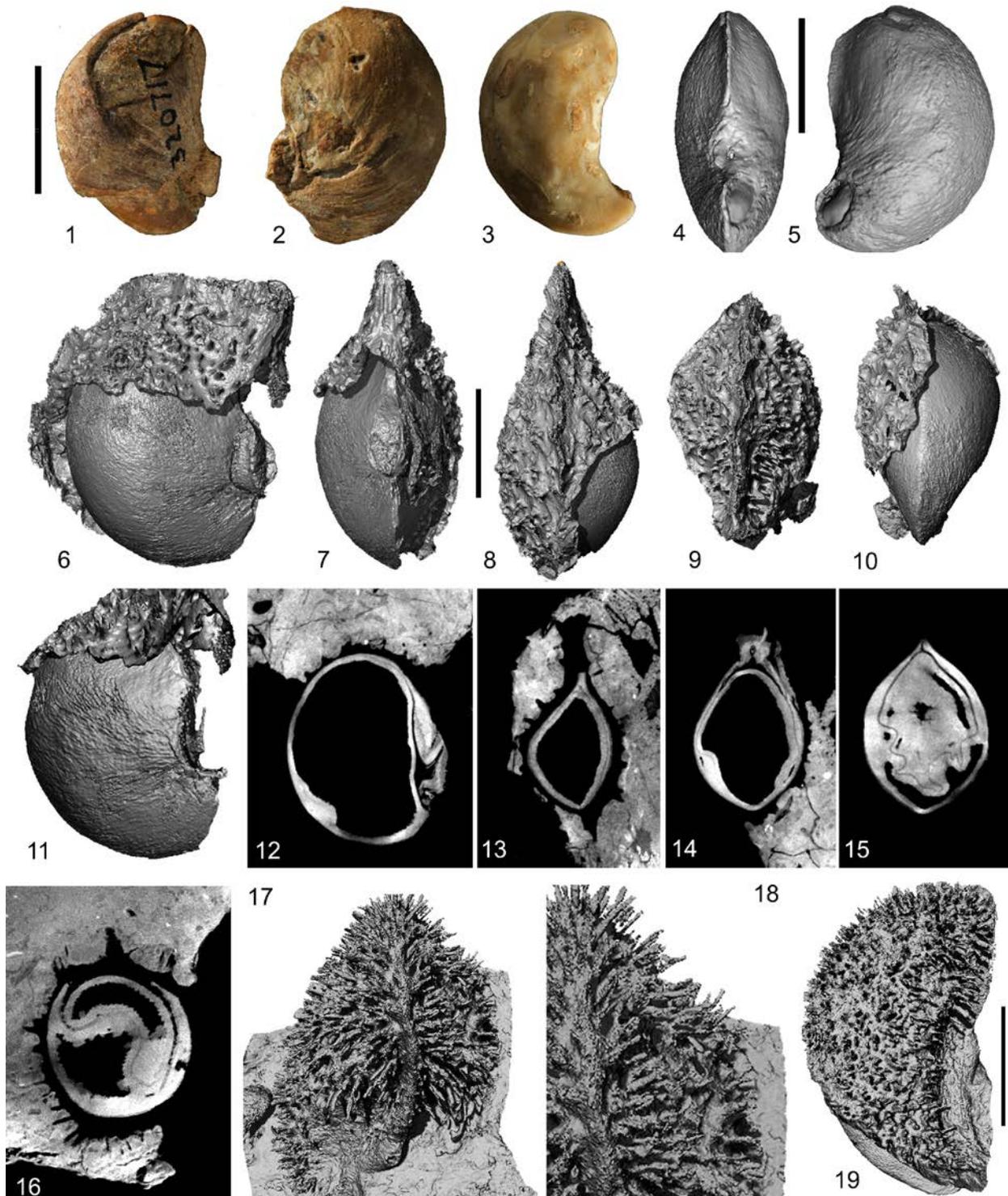
Plate 1. **1–19**. Disseminules of *Pseudoanacardium peruvianum* (Berry) comb. nov. **1–3**. Locule casts by reflected light, originals of Berry (1924, 1927). Scale bar = 1 cm; **1**. Lectotype from Berry 1924 in lateral view, with two wall layers exposed by fractures of the surface. USNM 320717; **2**. Specimen in lateral view showing protruding ventral attachment scar or placenta and curved surface striations. USNM 320713; **3**. Smooth interior cast, lateral view. USNM 320715; **4, 5**. Locule cast, surface rendering from CT scan. UF 603-70621. Scale bar = 1 cm; **4**. Ventral view showing keel in plane of bisymmetry; **5**. Lateral view, showing convex dorsal surface and concave ventral surface; **6–15**. Surface renderings and virtual sections of fruit with spiny ornamentation partially intact. UF 603-54895. Scale bar = 1 cm; **6**. Lateral view showing exposed locule cast with peg protruding on ventral side, and remains of spiny endocarp; **7**. Ventral view; **8**. Dorsal view; **9**. Apical view of locule cast with some adhering remains of spiny endocarp; **10**. Basal view; **11**. Lateral view as in fig. 6, but with external surface of "peg" rendered transparent, showing internal vascular strand (funicular or placental); **12**. Digital longitudinal section approximately in plane of bisymmetry, showing placental strand or funicle (left). Black part is airspace (including deteriorated endocarp wall); **13**. Transverse section at apical 1/3, showing spiny wall; **14**. Transverse section transecting ventral peg and vascular strand; **15**. Transverse section of specimen in figs 4, 5, showing thin wrinkled membrane of seed coat within. UF 603-70621; **16–19**. Digital section and isosurface renderings of UF603-54943; **16**. Transverse slice showing thickness of spiny fruit wall; **17**. Isosurface rendering, basal view; **18**. Same, enlarged; **19**. Lateral view, showing spines over lateral and dorsal surface, and relatively smooth ventral rib. Scale bar = 1 cm

Emended description. Chalcedony locule casts smooth-surfaced and cashew-shaped: reniform in overall form and rounded at one end and more pointed at other end, bisymmetrical, with keel in plane of symmetry, lensoidal to nearly circular in transverse outline. Locule casts 20–26 mm long, 8–15 mm wide (measured perpendicular to plane of symmetry), and 11–18 mm in dorsiventral depth. Fruit densely ornamented with spines except over raised area in ventral concavity. Excluding spines, fruits are 28–30 mm long, 11–14 mm wide (measured perpendicular to plane of symmetry), and 15.5–22 mm in dorsiventral depth. Spines 1.6 mm long, 0.2 mm wide, stiff and terete. Endocarp wall surrounding locule casts, represented by empty space between locule cast surface and surface of sedimentary fruit mold, 1–1.3 mm thick, excluding spines. Large circular scar (8.0 mm in diameter (or ca 1/3 of full length of locule) centered on midpoint of ventral concavity of locule cast, and aligned with ventral protrusion. Remains of a wrinkled seed coat preserved within locule cast of some specimens indicate one seed per fruit.

Lectotype. Berry (1924) did not designate a type for the species, but illustrated eight specimens (seven from Peru and one from Colombia). From these initial specimens we now designate the specimen of his figure 1 as lectotype (USNM 320717; refigured here, Pl. 1, fig. 1). The same specimen was also illustrated by Berry (1927) as Plate 20, fig. 1, when he first applied the name Belén to the locality. We agree that the other specimens that Berry figured, two of which are reillustrated here (Pl. 1, figs 2, 3), represent the same species.

Locality. The original specimens were collected by invertebrate paleontologist A.A. Olsson from a site indicated to be about 6 miles southeast of Parinas Point. We relocated the outcrops in 2010 (Manchester et al. 2012a) and obtained GPS readings of 4°44.946′S, 81°14.137′W (UF 602) and 4°44.966′S, 81°14.219′W (UF 603).

Additional specimens. The remaining syntypes of Berry 1924 (USNM 230713–320716) were studied, as were those of Berry (1929, Pl. 2, figs 1–4) at USNM and many specimens from the UF collection. Those with both external mold and locule cast include UF603-54875, 54881, 54884, 54891, 54894, 54895, 54900, 54902, 54941, 54943 and 54890; UF602-54742 (Figs 5A-C in Manchester et al. 2012a). Isolated locule casts: UF602-54739, 54740, 54741; UF603-70019, 70020, 70021, 70022, 70023, 70024, 70026 and 70027.

DISCUSSION

Pseudoanacardium is represented by spiny external molds and smooth internal casts. The internal casts, being silicified, are more resistant than the surrounding sediment, so these casts are often recovered as isolated specimens

Plate 2. 1–26. Disseminules of *Pseudoanacardium peruvianum* (Berry) comb. nov.; **1–10.** Cast and mold of fruit. UF603-54881. Scale bar = 1 cm; **1.** Dorsal surface of silica locule cast, still partially encased in sedimentary mold of fruit, reflected light; **2.** Same specimen, with locule cast removed, showing concavity of mold with outline of ventral smooth ridge surrounded by punctate surface indicating protrusion of spines, reflected light; **3.** Ventral view of virtual fruit cast partially covering silica endocarp cast. Surface rendering from CT scan; **4.** Locule cast from fig. 1, removed from matrix and viewed laterally, reflected light. Note large circular scar on ventral side (arrow). Reflected light; **5.** Same view of locule cast, surface rendering from X-ray data; **6.** Same, rotated 90° to show ventral surface with keel and large circular scar (arrows); **7.** Same as fig. 3, rotated 90° to show lateral view with portion of the spiny endocarp remaining, and smooth locule cast protruding; **8.** Same, flipped 180°, showing distinction between spiny ornamentation over most of fruit and smooth ventral area; **9.** Same with portion of endocarp digitally removed, revealing circular scar of locule cast within (arrow); **10.** Same specimen in apical view; (Supplemental Material: http://bomax.botany.pl/filmy/Acta_Palaeobot_58_1_Manchester_Suppl_UF_54881.mpg); **11–14.** Isosurface rendering of fruit with partially intact spiny endocarp. UF603-54890. Scale bar = 1 cm; **11.** Lateral view; **12.** Basal view. Note keel in plan of bisymmetry; **13.** Ventral view; **14.** Dorsal view. UF603-54890; (Supplemental Material: http://bomax.botany.pl/filmy/Acta_Palaeobot_58_1_Manchester_Suppl_Anac_54890.mpg) **15–17.** Digital section and isosurface renderings of UF603-54941; **15.** Transverse slice showing spines of endocarp protruding into sediment (left side). Space between locule cast and endocarp mold represents original thickness of endocarp; **16.** Surface rendering with locule cast partially covered by remains of spiny endocarp, basal view; **17.** Surface rendering, lateral view; **18–23.** Surface renderings of UF603-54891; **18.** Lateral view with somewhat eroded (spines rounded), endocarp over upper half, and smooth locule cast exposed in lower half; **19.** Same view with covering portion of endocarp removed to reveal apical part of locule cast with its circular scar (arrow); **20.** Oblique ventral view showing delimitation between smooth ventral surface and ornamented lateral and dorsal surface of endocarp; **21.** Dorsal view of endocarp with eroded spines; **22.** Dorsal view of locule cast; **23.** Apical view of endocarp; **24.** Sedimentary mold showing punctate surface indicative of spiny fruit, from which images in 15–17 were taken. Reflected light. UF603-54941. Scale bar = 1 cm; **25.** Surface rendering of spiny fruit in ventral view with smooth, fusiform ventral rib showing median groove (arrow). UF603-54894. Scale bar = 1 cm; **26.** Longitudinal fracture of sedimentary mold from fig. 24, revealing spines in longitudinal view. UF603-54941. Scale bar = 3 mm

eroded out of the sediment without preservation of the external layers. Such casts (e.g. Pl. 1, figs 1–5) were the only specimens available to Berry (1924, 1927, 1929). However, we were fortunate to obtain more complete specimens with both the external mold and internal cast preserved. There is some air space between the cast and mold, such that the cast can move around slightly within the mold when shaken by hand. This space represents the original
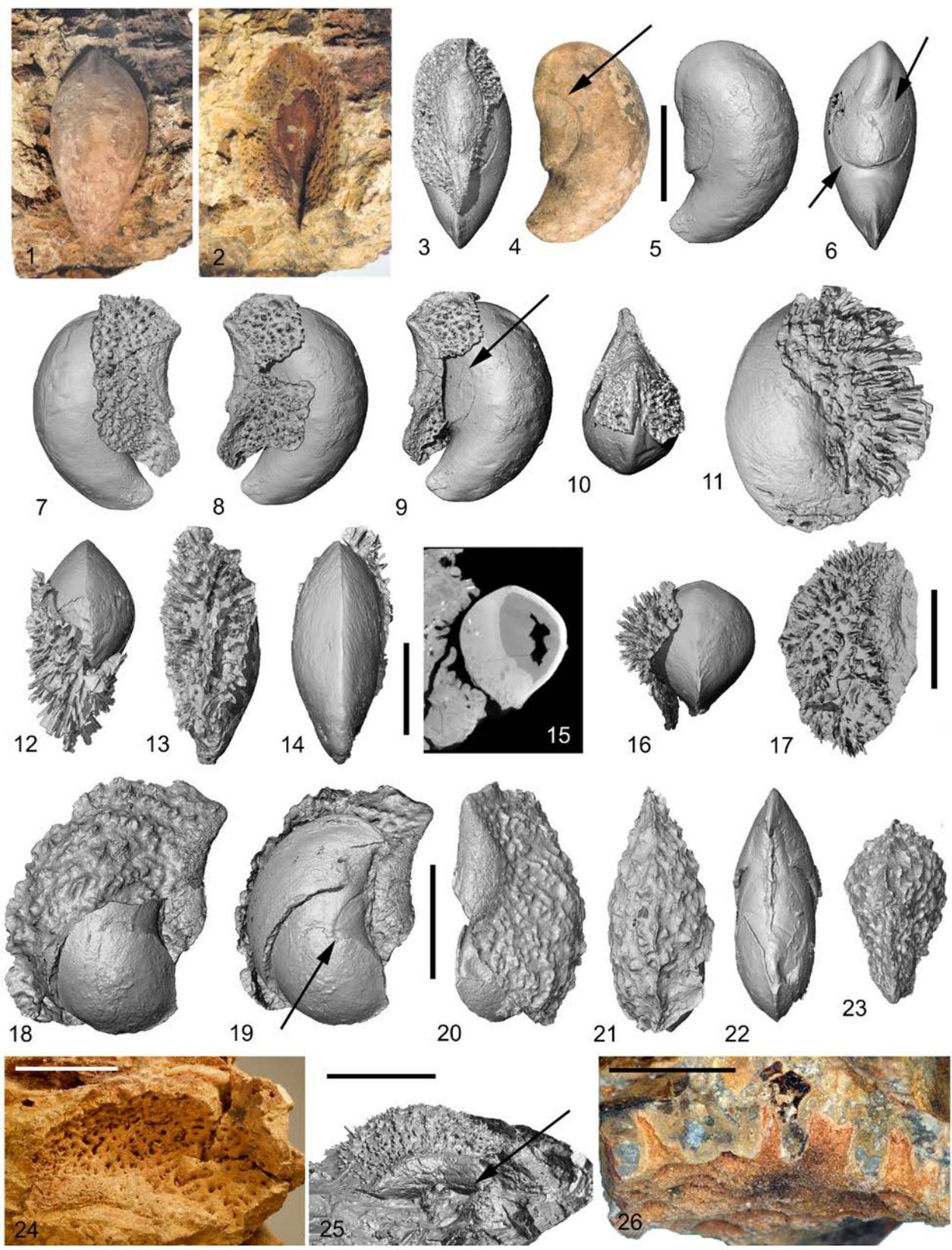
wall that was impervious to mineralizing fluids, which has since deteriorated.

The endocarp spines are represented only by molds or impressions in the sediment and are seen as scattered cylindrical holes on the surface of the sedimentary molds (Pl. 2, figs 2, 24, 26). Prior attempts to produce silicone casts of these molds were unsatisfactory: although silicone penetrated the spines under vacuum, the tips of the cured silicone spines broke off during attempts to separate these artificial casts from the sedimentary molds. Digital surface renderings from CT scan x-ray data provided a superior method of documenting the morphology and distribution of these spines (e.g. Pl. 1, figs 6–19; Pl. 2, figs 7–23, 25, and movies in Supplemental Material). The spines protrude from all over the surface, except for a raised, fusiform area along the ventral concavity. In some specimens the spines were shorter and blunter (e.g. Pl. 2, figs 7, 20 vs. Pl. 1, fig. 17; Pl. 2, fig. 12), apparently due to abrasion prior to deposition. They appear to have been rigid and rather brittle because they are observed to be broken but not bent within the sediment.

Berry (1924, 1927) was convinced by the available specimens that the fossils were those of cashews and included this hypothesis in his descriptive text: "Nuts reniform in shape, with a thick shell which is missing in a good many of the specimens, but is more or less preserved in several. The surface of the latter show the same faint, oblique markings due to the underlying fibrous, acrid, secretory layer, exactly as in the nuts of *Anacardium occidentale*, and there is exactly the same wrinkling of the outer coat in the region of the sinus as in the recent species. Where the outer, ligneous coat had rotted before silicification, or been subsequently abraded, the inner coat shows identical oblique channelings parallel with the lower margin of the nut exactly as is shown by the secretory layer of the modern nut."

Indeed, the variously preserved layers of the fossil specimens show some similarities to the structure of an *Anacardium* fruit. However, similarities based on degraded, poorly preserved wall layers with limited anatomical detail cannot be taken as proof of identity. Cashews have their pedicel and attachment on the convex surface of the fruit somewhat removed from the rounded extremity. The fossils instead have their only obvious attachment scars in the concave region of the fruit

body (Pl. 1, figs 2, 6, 11, 12). The prominent keel in the plane of symmetry of the fossil (e.g. Pl. 1, figs 4, 9, 10; Pl. 2, figs 6, 10, 22) also contrasts with the smoothly rounded outline in the plane of symmetry in *Anacardium* fruits. The newly recovered specimens documenting the outer ornamentation of stiff, needle-like spines support the conclusion that these fossils have nothing to do with Anacardiaceae.

The morphological features observed on the ventral side of the disseminule vary depending on the layer that is exposed. The innermost surface, when preserved, shows a circular scar centered on the midpoint of the ventral concavity (Pl. 2, figs 4–6, 9, 19). It is relatively large, ca 1/3 the length of the locule, and the area that it delimits is smooth, as is the rest of the surface outside the circular mark. This might be a hilar scar, but if so, it is unusually large; or it might be a funicular or placental scar. Its morphology does not carry through to the external layers; however, a peg-like ventral protrusion (Pl. 1, figs 1, 2, 6, 7) is approximately superimposed over the center of the circular mark on specimens with the next successive layers preserved within the fruit. The ventral protrusion is elliptical in cross section and is narrower than the circular scar beneath it. A longitudinal section through the peg in the fruit's plane of bisymmetry reveals a vascular channel (Pl. 1, figs 11, 12), which may represent the funicular strand or vasculature between the pedicel and placenta. The exposed surface of casts with the ventral peg preserved commonly is diagonally striate (e.g. Pl. 1, fig. 2). External to this striate surface is the layer occupied by empty space representing the former endocarp wall with its spiny ornamentation. The ventral region of the endocarp is marked by a prominent fusiform raised rib (Pl. 1, fig. 19, Pl. 2; figs 2, 3, 7–9, 17, 19) which lacks spines and has a narrow median groove (Pl. 2, figs 20, 25). Spines emanate from all surfaces of the endocarp except for this portion of the ventral concavity. It is uncertain whether there was a substantial mesocarp and exocarp external to the spiny wall. Soft tissues are not preserved for other fruits known from the Belén locality (e.g. Annonaceae, Vitaceae), so if such tissue was present in *Pseudoanacardium* we would not expect it to have been preserved.

The fruit spines appear to have been straight and needle-like, providing a protective function

against herbivory. They are not curved, hooked or barbed at the tips as would be expected in the case in epizoochorous adhesively dispersed diaspores (Sorensen 1986). Superficially similar spiny fruits include pods of the nickernut, *Guilandina bonduc* (Caesalpinaceae). Those fruits are also spiny and bilaterally symmetrical but often have two rather than just one seed per fruit, and the seeds are globose and lack the large circular scar seen in the fossils. Another legume, *Centrolobium*, is known for its very large *Acer*-like winged fruits, but also is armed with spines over the proximal seed-bearing end of the pod; this differs from *Pseudoanacardium* by both the large size and the prominent samaroid wing. Endocarps of *Parabaena megalocarpa* (Menispermaceae) were also compared. Although similar in size and spininess, the locules of *Parabaena* and other members of that family are crescent-shaped in cross section, unlike the fossils (e.g. Jacques 2009). Spiny fruits of other taxa like *Datura* (Solanaceae) are readily distinguishable by their multiple locules. The spiny fruits of *Xanthium* (Asteraceae) are straight rather than curved. It may be that these fruits conform to Sapindaceae, which includes spiny fruits in several extant genera (e.g. *Jagera, Nephelium, Paulinia, Xerospermum*; Doweld 1996). However, we have not seen evidence of the coiled or folded embryo that would be expected. We were unable to unravel the familial affinities of *Pseudoanacardium* through these kinds of comparisons. The few specimens with seed coat preserved (Pl. 2, figs 15, 16) seem to indicate a seed with copious endosperm and not with a large embryo. If there were a pair of large cotyledons of the kind seen, for example, in legumes, we would expect their outline to be evident with this nature of preservation.

CONCLUSIONS

Although the newly revealed details of these fossils clearly distinguish them from Anacardiaceae and confirm that the fruits are not cashews, the actual affinities remain uncertain, and it is likely that they represent an extinct genus for which familial relationships remain to be determined. While it is unsatisfying to leave these with undetermined status, we feel it important to remove any suggestion that these fossils contribute to an understanding of the evolution and biogeographic history of *Anacardium*. Given that *Anacardium* is natively distributed in Central and South America today, it is somewhat surprising that fossil occurrences of *Anacardium* fruits are not yet confirmed from South America, although fruits complete with the diagnostic hypocarp are known from the Middle Eocene of Germany (Manchester et al. 2007). Recently, however, anatomically preserved wood from the late Eocene of Sexi, Peru, has been identified as *Anacardium* (Woodcock et al. 2017).

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