Integrated Chronology, Flora and Faunas, and Paleoecology of the Alajuela Formation, Late Miocene of Panama

Bruce J. MacFadden*, Douglas S. Jones, Nathan A. Jud, Jorge W. Moreno-Bernal, Gary S. Morgan, Roger W. Portell, Victor J. Perez, Sean M. Moran, Aaron R. Wood

1 Florida Museum of Natural History, University of Florida, Gainesville, Florida, United States of America, 2 Smithsonian Tropical Research Institute, Ancon, Panama, 3 New Mexico Museum of Natural History, Albuquerque, New Mexico, United States of America, 4 Department of Geological Sciences, University of Florida, Gainesville, Florida, United States of America, 5 Department of Biology, University of Florida, Gainesville, Florida, United States of America, 6 Department of Geological & Atmospheric Sciences, Iowa State University, Ames, Iowa, United States of America

* bmacfadd@flmnh.ufl.edu

Abstract

The late Miocene was an important time to understand the geological, climatic, and biotic evolution of the ancient New World tropics and the context for the Great American Biotic Interchange (GABI). Despite this importance, upper Miocene deposits containing diverse faunas and floras and their associated geological context are rare in Central America. We present an integrated study of the geological and paleontological context and age of a new locality from Lago Alajuela in northern Panama (Caribbean side) containing late Miocene marine and terrestrial fossils (plants, invertebrates, and vertebrates) from the Alajuela Formation. These taxa indicate predominantly estuarine and shallow marine paleoenvironments, along with terrestrial influences based on the occurrence of land mammals. Sr-isotope ratio analyses of in situ scallop shells indicate an age for the Alajuela Formation of 9.77 ± 0.22 Ma, which also equates to a latest Clarendonian (Cl3) North American Land Mammal Age. Along with the roughly contemporaneous late Miocene Gatun and Lago Bayano faunas in Panama, we now have the opportunity to reconstruct the dynamics of the Central America seaway that existed before final closure coincident with formation of the Isthmus of Panama.

Introduction

The late Miocene was an important time to understand the biodiversity dynamics in the Neotropics. No place within this region was more important than Panama because it functioned as both a gateway and barrier to, respectively, interoceanic and intercontinental dispersal of Neotropical biotas. Despite this importance, until recently our knowledge of late Miocene biotas has been restricted to marine faunas such as the hyperdiverse, Gatun Formation, which preserves an excellent record of both invertebrates [1] and chondrichthysans [2]. In contrast to the
marine record, up to now no terrestrial macrofossils have been reported in Panama from the time interval between the early Miocene of the Panama Canal localities [3] and the late Pleistocene of the Azuero Peninsula [4]. The apparent lack of terrestrial sedimentation, as evidenced by the paucity of outcrops during the late Miocene portion of this interval, likely resulted from the rapid uplift and consequent erosion during the formation of the isthmus [5]. This hiatus of almost 19 million years in the non-marine fossil record of Panama is nevertheless critical to understanding the terrestrial faunal dynamics before and after the final closure of the Central American Seaway and rise of the Isthmus during the Pliocene.

The Miocene proboscidean *Gomphotherium*, which was recently described from Lago Alajuela (previously Lake Madden), begins to fill in this critical hiatus [6]. As it is known from elsewhere in Holarctica [7], the genus *Gomphotherium* has a fairly long temporal range, between about 15 to 5 Ma, and it is this interval that we have previously assumed for the age of the Alajuela Formation. Over the past two years we have discovered the original site where *Gomphotherium* was collected in 1959 and conducted further field work to recover additional fossils from the sedimentary units that crop out along the shore and islands of Lago Alajuela, particularly during times of lowered lake level.

The purpose of this report is to present a preliminary description of the invertebrate and vertebrate faunas and floral remains collected from Lago Alajuela, describe the physical stratigraphy, present new Sr-isotope ratio age determinations, and discuss the significance of these data in light of our understanding of the biodiversity and paleobiogeography of the late Miocene of Panama. We realize that certain aspects of this paper are incomplete, most notably the faunas, which still represent only a small fraction of the overall ancient biodiversity. Nevertheless, the implications of these occurrences, plus the new Sr-isotope dates, have considerable significance for an area that is of great current interest in terms of New World tropical (paleo) biology.

**General Background and Previous Geological Studies**

Sediments referred to the Alajuela Formation were deposited in the Panama tectonic basin [8] and crop out along the shore of Lago Alajuela in the Panama Province (Fig 1) at a general lat. 9.2124˚ N, long. 79.5936˚ W and an elevation of about 80 m. The fossil localities, including the initial *Gomphotherium* discovery in 1959 by John Turner, occur along the shores and adjacent small islands of the southwestern portion of the lake (Table 1), less than 1 km from the Chagres National Park Headquarters, which is also the general site of the now defunct Madden Lake Boy Scout Camp [9]. Woodring [10] mapped relevant outcrops along the shore of Lake Madden as pertaining to the lower to middle Miocene Caimito Formation (including the Alajuela Member), whereas Woodring et al. [11] mapped these as the upper member of the upper middle Miocene Alajuela Formation, consisting of tuffaceous sandstones, calcareous sandstones, and limestones. In these maps, the Alajuela Formation is depicted (in the legend) to underlie the well-known Gatun Formation, although the direct superpositional field relations to confirm these relative ages are not well supported. In fact, the Sr-ratio age determinations presented here address the relative ages of these formations, as will be discussed below. Our geological map, modified from Woodring et al. [11], is presented in Fig 2.

**Materials and Methods**

This study integrates three domains of investigation: i.e., lithostratigraphy, Sr-isotope dating, and paleontology. The lithostratigraphic work was done in the field by measuring and describing stratigraphic sections and locating these via GPS. With regard to Sr-ratio dating, fossils were collected from our composite measured section as documented below. Powdered low-
magnesium calcite samples were drilled from the interior of each specimen using a hand-held Dremel tool with a carbide dental burr. Approximately 0.01 to 0.03 g of powder was recovered from each fossil sample and these were analyzed according to standard techniques [12]. The powdered samples were dissolved in 100 μl of 3.5 N HNO₃ and then loaded onto cation exchange columns packed with strontium-selective crown ether resin (Eichrom Technologies, Inc.) to separate Sr from other ions. Sr isotope analyses were performed on a Micromass Sector 54 Thermal Ionization Mass Spectrometer equipped with seven Faraday collectors and one

Table 1. List of location sites from which the fossils described in this report were collected from the upper Miocene Alajuela Formation, Lago Alajuela, Panama (UF VP general locality YPA088; lat. 9.2124°, long. -79.5936°).

| Site Designation* | Latitude (°) | Longitude (°) |
|-------------------|--------------|---------------|
| 630041            | 9.2159       | -79.5972      |
| 630042            | 9.2156       | -79.595       |
| 630043            | 9.2141       | -79.5942      |
| 630044            | 9.2130       | -79.5932      |
| 630045            | 9.2149       | -79.5946      |
| 630046            | 9.2223       | -79.5978      |
| 630048            | 9.2119       | -79.5929      |
| 630049            | 9.2114       | -79.5919      |
| 630050            | 9.2200       | -79.5888      |
| 630051            | 9.2107       | -79.5915      |
| 630052            | 9.2090       | -79.5913      |
| 630053            | 9.2164       | -79.6012      |
| 630061            | 9.2160       | -79.5983      |
| 630066            | 9.2161       | -79.5987      |

*The relative stratigraphic positions of these sites are also listed on Fig 3.
Daly detector in the Department of Geological Sciences at the University of Florida. Sr was loaded onto oxidized tungsten single filaments and run in triple collector dynamic mode. Data were acquired at a beam intensity of about 1.5 V for $^{88}\text{Sr}$, with corrections for instrumental discrimination made assuming $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$. Errors in measured $^{87}\text{Sr}/^{86}\text{Sr}$ are better than $\pm 0.00002$ (2σ), based on long-term reproducibility of NIST 987 ($^{87}\text{Sr}/^{86}\text{Sr} = 0.71024$). Age
Fig 3. Composite lithostratigraphic section of the Alajuela Formation as exposed in northeastern Chagres National Park, Panama along Lago Alajuela. Key lithologic intervals and units described in the text are marked to the left. Relevant fossil
estimates were determined using the Miocene portion of Look-Up Table Version 4:08/03 associated with the Sr isotopic age model [13].

With regard to paleontology, invertebrate, plant, and vertebrate fossils were collected from the individual measured sections and integrated into the composite section described below. Fossils were collected under permit 2014–21 issued by the Ministerio de Comercio e Industrias, Dirección Nacional de Recursos Minerales, Republic of Panama. Fossils were collected mostly by surface prospecting; although matrix was collected in an attempt to recover microfauna, this proved unsuccessful. The identification of some invertebrate fossils was enhanced by making casts of some moldic mollusks. Fossils were trimmed with a rock saw, cleaned, and a room temperature vulcanizing (RTV) silicone rubber was vacuumed into the external molds. Once cured (12 hours), the silicone rubber peels were removed for examination and later identification. In order to study fossil wood specimens, thin-sections were produced using standard techniques [14] along transverse, tangential, and radial planes. A total of 41 specimens were examined in transverse section to evaluate the quality of preservation and to distinguish palms from dicots. Twenty-four of dicot woods were sectioned along tangential and radial planes for further identification. We used the InsideWood Database [15, 16] and Metcalfe and Chalk [17] to identify the fossils to families.

The fossils described below are contained within three paleontological collections at the Florida Museum of Natural History, University of Florida, and are referred to by the acronyms UF-I (Invertebrate Paleontology), UF-P (Paleobotany), and UF-V (Vertebrate Paleontology). The relevant data for these can be retrieved on-line at www.flmnh.ufl.edu.

**Litho- and Biostratigraphy of the Alajuela Formation**

The Alajuela Formation includes a >25 m-thick basal package of interbedded, clast-supported conglomerates and litharenite sandstones that grades into an ~85 m-thick package of calcareous sandstones and calcarenites, representing a transition from tide-dominated, potentially estuarine, coastal environments to wave-dominated, shallow-water carbonate environments [10,18]. The 82 m-thick composite section (Fig 3), measured in proximity to the fossil localities on the southern extent of Lago Alajuela (Table 1), is subdivided into three distinctive lithological intervals to summarize major facies transitions during this transgression.

Within the basal-most Interval 1, laterally-extensive horizons of amalgamated conglomerate lenses fine upwards into fine-medium grained sandstones (Unit A of Fig 3), truncated by erosional contacts with overlying units. The best exposed amalgamated conglomerate (near the 15 m level in the composite section) exhibits substantial variability in thickness (1–6 m) with an average thickness of ~3 m. The amalgamated conglomerates are generally clast-supported, but locally matrix-supported, with the coarse grain fraction rarely exceeding 5 cm in diameter. Weathered exposures of the conglomeratic horizons erroneously appear matrix-supported due to diagenetic dissolution of aragonitic shell material. Prior to such dissolution, the coarse fraction would likely have been dominated by mollusk shells, whereas well-rounded, pebble- to cobble-sized volcanic fragments (welded tuff and andesite) and silicified woods comprise the minor component of the coarse fraction. Fossil invertebrate remains primarily consist of internal and external molds of mollusk shells preserved in fine-grained sand matrix as well as some original calcitic shell material of scallops and oysters (also see discussion below). The amalgamated conglomerates contain the most abundant vertebrate fossils, both
well-preserved remains of marine vertebrates (e.g., sharks) and highly weathered remains of terrestrial vertebrates.

The conglomerate exhibits a dm-scale gradational contact with a poorly sorted, fine-medium grained sandstone with abundant lithic and feldspar grains. The sandstone is locally tuffaceous and exhibits, in some exposures, low-angle, dm-thick bedforms that are internally massive and dip perpendicularly to the overall attitude of the Alajuela Formation. Otherwise, the sandstone appears massive and highly bioturbated, containing a lower density of molluscan molds than the underlying conglomerate. Original molluscan shell material and marine vertebrate fossils are present but rare. No terrestrial vertebrate remains have been found within the sandstone horizons of Unit A.

One exposure of Interval 1, within the 9–14 m levels of the composite section, includes a moderately sorted, fine-medium grained tuffaceous sandstone with distinctive mm- to cm-thick horizontal bedding and wavy laminations (Unit B of Fig 3). Scour and fill structures with dm-scale widths and occasional mud drapes are present near the erosional contact with the underlying Unit A lithologies. No body fossils are present in this unit, but rare and well-preserved ichnofossils, primarily Conichnus and vertically-oriented Ophiomorpha (Fig 4), are preserved that cut across and deform bedding horizons. The infilling of these burrows consists of the same fine tuffaceous sand of overlying horizons.

The base of Interval 2 exhibits a highly irregular, erosional contact with either a poorly sorted, bioturbated sandstone of Unit A lithologies or a laterally discontinuous unit of clast-supported conglomerate (Unit C in Fig 3). This interval typically occurs well above lake levels in the study area and is covered by vegetation. Consequently, continuous fresh exposures exhibiting diagnostic sedimentary structures are relatively rare. The dominant lithology in Interval 2 appears to be a well-cemented, fine-grained litharenite that coarsens upwards into a medium-grained sand capped by a shell lag horizon of fragmented bivalves and gastropods.
(Unit D of Fig 3). The density of shell fragments at the top of the coarsening-upwards sequences locally approaches that of a coquina. A minimum of three coarsening-upward sequences is preserved in Interval 2, and although the litharenite appears massive in most exposures, trough cross-bedding and low-angle planar cross-bedding are evident locally. The base of Interval 3 is marked by a cm-scale gradational contact between an underlying shell lag in Interval 2 and an overlying fine-grained calcareous sandstone with lithics and occasional trough cross-bedding and ripple marks (Unit E of Fig 3). A calcarenite occasionally interbedded with sandy limestone (Unit F of Fig 3) occurs stratigraphically above, separated from the underlying calcareous sandstone by an irregular, erosional contact. Sedimentary structures vary within the calcarenite from trough cross-bedding to wavy bedding to low-angle planar cross-bedding, suggesting substantial changes in flow velocities at the time of deposition. The lithologies in Unit F were originally described by Woodring [10] as the Alhajuela [sic.] Sandstone Member of the Caimito Formation. However, based on the age constraints presented below for the Alajuela Formation, this attribution to the late Oligocene—early Miocene Caimito Formation is no longer supported. The stratigraphic thickness of Interval 3 was not measured in the present study but was reported by Woodring [10] as being approximately 85 m.

Interval 1 is tentatively interpreted as sediment deposited in the distal area of a tide-dominated estuary. The amalgamated conglomerate horizons with variable thickness likely represent lag deposits within tidal channels whereas the overlying bioturbated sandstones represent tidal sand bars with lateral accretionary surfaces (i.e., the low-angle, dm-thick bedforms described above in Unit A). It should be noted that we have no unequivocal evidence for net landward movement of sediment, a defining characteristic of estuarine deposits in the sedimentary record [19], other than the overall transgressive sequence in the Alajuela Formation. However, the transition from 1) channelized conglomerates to 2) laterally-accreting sand bodies to 3) horizontally-bedded well-sorted sand, all with marine body and trace fossils, is consistent with a transition from outer estuarine sand bars to upper flow regime sand flats in the tide-dominated estuary model of Dalrymple et al. [19]. The association of horizontal bedding, cut and fill structures, and Ophiomorpha and Conichnus ichnofossils within Interval 1 furthers supports an estuarine depositional environment [20].

Deposits within Intervals 2 and 3 in the composite section are interpreted to have been deposited in wave-dominated nearshore environments. A precise depositional interpretation for Interval 2 lithologies, however, is problematic without well-exposed diagnostic sedimentary structures. The presence of fine to medium grained litharenites with some evidence of trough cross-bedding and low-angle planar cross-bedding tentatively suggests an upper to middle shoreface depositional environment, but better exposures on the northern shore afforded by a drop in lake levels are necessary to verify this interpretation. The Interval 2 to 3 transition is marked by an increase in carbonate material that culminates in a well-sorted calcarenite, indicating a shift from terrestrial-sourced sediment to marine-derived sediment without a concurrent increase in water depth.

Most of our fossil samples have been collected from outcrops of the Alajuela Formation along the southwestern shores of Lago Alajuela. These localities are georeferenced and can be placed stratigraphically within our composite measured section (Fig 3). In addition to these localities, however, several other fossil localities from outcrops that appear to be correlated to the Alajuela Formation were sampled from Isla Vigia and other smaller islands within a few km west of our stratigraphically controlled localities. These localities yielded fossil invertebrates, wood (Parinariosylon sp.), and vertebrates including turtles and several taxa of shark teeth, dominated by the diagnostic Carcharocles megalodon. We realize that although the island localities are not stratigraphically calibrated like those on shore, they nevertheless attest to the additional paleontological potential of the Alajuela Formation.
So far as lithostratigraphic studies in the field demonstrate, no suitable volcanic units crop out within our measured sections of the Alajuela Formation. The occurrence of original shell material from marine fossils therefore provided an opportunity to determine the age of the unit using $^{87}\text{Sr}/^{86}\text{Sr}$ geochronology. Mollusk shells (scallops and oysters) were collected from a ~2 m thick interval about 10 to 12 m above the base of the composite section and below a prominent fossiliferous conglomerate (Fig 3). As will become important below in the discussion with respect to the age of diagnostic fossils, this zone also approximates the levels where the terrestrial vertebrates were collected.

With the exception of sample LKA-1, which is interpreted to represent an outlier due to diagenetic alteration observed in shell cross-sections, ratios were tightly clustered between 0.708850 and 0.708920 (Table 2). Comparison of the mean ratio with the global seawater $^{87}\text{Sr}/^{86}\text{Sr}$ curve for the Neogene [13] indicates a late Miocene age of 9.77 ± 0.22 Ma for this portion of the Alajuela Formation (Fig 5). The strontium dates also confirm a late Miocene age for the associated vertebrate fossils including the biochronologically informative horses and proboscidean Gomphotherium sp.

### Sr-isotope Ratio Age Determinations

Silicified woods occur as rounded to subangular clasts with low sphericity that often occur as float. These clasts tend to be concentrated in the lower part of the section and occasionally found as part of the coarse-fraction in Interval 1 (Fig 3). Based on 60 samples that we collected, the mean wood clast length is 7.3 cm ($s = 2.4$ cm) with an observed range of 3 cm to 29 cm. (Rounded chert clasts up to about 4 cm long also occur within the formation.)

Three of the 41 specimens examined in transverse section are palms, including at least two types: a one-vessel palm and a two-vessel palm. Although the assemblage is very rich, none of the dicot wood types (species) are represented by more than one specimen. A complete description and taxonomic allocation of each wood type is beyond the intended scope of this report; however, at this point some of the specimens can be identified to family level (Fig 6). Based on the characters preserved so far, the families recognized are Annonaceae, Phyllanthaceae, Fabaceae, Humiriaceae, Malvaceae, and Sapindaceae (Fig 6; S1 Table). Chrysobalanaceae is also represented in the Alajuela Formation, but from a different locality (630050) of unknown stratigraphic position relative to our measured sections. Each of these families is found in tropical forests in Panama today [21, 22]. All of the specimens are diffuse-porous,
and only two (UF-P 63106 and UF-P 63120) have indistinct growth rings marked by narrow, thick-walled fibers, that may be annual based on the width and complacency. Some of the dicot woods examined in transverse section (six out of 38) have very large (>200 μm) mean vessel diameters, a character state typical of large canopy trees in tropical forests [23–25]. Most of the woods examined in longitudinal section (22 out of 24) have simple perforation plates and many of the woods (11 out of 38) have elaborate conformations of axial parenchyma (e.g., aliform, confluent, or wide bands). The rarity of growth rings, the presence of very large mean vessel diameters, abundant axial parenchyma, and the presence of both one- and two-vessel palms are all features found in taxa typical of Neotropical moist forests and rainforests [23, 24, 26–28].

The rounded, apparently rolled and abraded nature of the silicified woods in the conglomerates and sandstones of the Alajuela Formation raises the question whether these fossils represent silicic clasts that were reworked from (an) earlier depositional episode(s), or were contemporaneous with the vertebrate fauna, and transported as float before becoming water-logged, sinking, and then replaced with silica. In a manner similar to the recent comparison of Panama fossils [29], 77 rare earth element (REEs) samples were analyzed and compared for the invertebrate, vertebrate, and wood fossils and sediments from the Alajuela Formation. In addition, we analyzed fossil woods from the lower Miocene Cucaracha Formation in the southern Panama Canal Basin [3, 30, 31] and from the Ocú area on the Azuero Peninsula, Panama [32]. Typically, when taphonomically mixed fossils form during different sedimentary cycles, they demonstrate different REE patterns [33] that allow temporal discrimination. This, however, was not the case in our analyses, and the REE results were equivocal, i.e., they did not discriminate fossils within the Alajuela Formation as well as in comparison with the Cucaracha Formation and Ocú area woods. Although we present the REE data in S1 Table, we do not report these within the text because of the ambiguous outcome of the analyses. Regardless of the ambiguous results of the REE data with regard to the age of the fossils, other information can be brought to bear with regard to the Alajuela Formation woods.

Fig 5. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of eight shells analyzed from the Alajuela Formation yielding an age of 9.77 ± 0.22 Ma based on interpolation from McArthur et al. [16].

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Autochthonous and parautochthonous assemblages of fossil wood preserved as charcoal and calcium-carbonate permineralizations from the Cucaracha Formation have lower species richness and higher evenness than the Alajuela Formation assemblage; but the same families are present in both units [30, 31, 34, 35], as well as older Miocene palynofloras [36–39].

Fig 6. Cross sections of selected woods from the Alajuela Formation showing the variation in vessel diameter, vessel frequency, and conformations of axial parenchyma (e.g., narrow bands, absent/rare, uniseriate bands, wide bands, aliform/confluent). A. UF-P 63102 Malvaceae type a; B. UF-P 63103 Arecaceae; C. UF-P 63104 Malvaceae type b; D. UF-P 63105 Salicaceae/Rubiaceae; E. UF-P 63106 undet. angiosperm; F. UF-P 63109 Annonaceae; G. UF-P 63112 undetermined angiosperm; H. UF-P 63124? Phyllanthaceae; I. UF-P 63133 Humiriacea/son sp. (Humiriaceae); J. UF-P 63134 Sapindaceae; K. UF-P 63135 Ficus sp. (Moraceae); and L. UF-P 63146 Fabaceae.

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similarity of forest composition leaves open the possibility that the woods were reworked from lower Miocene deposits. On the other hand, the rarity of other clasts as large as the fossil woods in the conglomerates and coarse to medium sandstones of Interval 1 suggests the possibility that there may not have been enough energy for transporting relatively large silicified wood clasts. This therefore supports the hypothesis that the woods were transported as float prior to silicification and represent the vegetation roughly contemporaneous with the co-occurring fauna.

In summary, it is unclear whether the obviously rolled and abraded wood specimens were formed at the same time as the other fossils collected from the Alajuela Formation; however, their original age before reworking is likely constrained between about 21 Ma [40] and ~9.8 Ma (age of the Alajuela Formation; see above). Considering all the available evidence, we hypothesize that they are most likely to be of late Miocene age. Thus, these woods provide a glimpse of the forest community during the Miocene in the New World tropics, and help to demonstrate the continuity of tropical forest communities in Panama since that time.

In summary, the woods provide a glimpse of the forest community during the Miocene in the New World tropics, and help to demonstrate the continuity of tropical forest communities in Panama since that time.

Invertebrate Fauna

Two types of preservation commonly occur in the Alajuela Formation invertebrate fossils from Lago Alajuela: 1) body fossils whose hard parts originally consisted of calcite (e.g., bryozoans, scallops, oysters, decapods, and echinoderms) and 2) more commonly, internal and external molds of organisms whose hard parts originally consisted of aragonite (corals and the majority of mollusks). Representative photographs of mollusks, arthropods, and echinoderms are presented in Figs 7 and 8.

Phylum Cnidaria

Scleractinian corals are conspicuously absent from all localities indicating they were probably not present in this area at the time of deposition. Corals are typically found in normal marine conditions but do not tolerate the influx of freshwater.

Phylum Bryozoa

Only six lots of cheilostome and two lots of cyclostome bryozoans were recovered from the deposits of Lago Alajuela. Most were poorly preserved, either leached or in one case moldic. All are encrusting forms; no erect forms were present. The majority of zoaria were collected from locality 630043 and these consisted of an indeterminate cyclostome (UF-I 261059), as well as one confidently identified to the Family Plagioeciidae (UF-I 261079). An unidentified cheilostome (UF-I 261057) and three lots of the genus *Chaperia* (UF-I 261058, 261060, 261061) were found as well. One specimen (UF-I 261122) was collected at locality 630053 and, although highly-weathered, was determined to be *Hippoporidra* sp. One moldic cheilostome (UF-I 263314) was found at locality 630048.

Phylum Brachiopoda

One genus of brachiopod was discovered in the Alajuela Formation sediments along Lago Alajuela; the lingulid *Glottidia* sp. (UF-I 264953). The exposed valves are small (< 11 mm) and all were collected in situ at locality 630066. Living *Glottidia* is commonly observed in tropical and subtropical marine environments in intertidal to shallow subtidal zones [41], although Emig [42] suggests the intertidal zone is not the most optimal habitat for the genus because it is quite intolerant of lower salinity from freshwater influx.
Fig 7. Selected examples of late Miocene moldic mollusks (originally aragonitic-shelled) from the Alajuela Formation, Lago Alajuela, Panama. Room temperature vulcanizing (RTV) silicone rubber was vacuumed into external molds to provide peels in order to facilitate identification. A-C. UF-I 263319, *Turbinella* sp., A. external mold—apertural view, B. peel, C. internal mold. D. UF-I 263212, Cypraeidae, internal mold—apertural view. E. UF-I 263119, *Conus* sp., internal mold—apertural view. F-G. UF-I 263242, *Panopea* sp., F. external mold, G. peel. H-I. UF-I 264011, Turritellidae, H. internal mold—apertural view, I. peel. J-K. UF-I 263100, *Melongena* sp., J. external mold—dorsal view, K. peel. L-M. UF-I 263231, *Calyptraea* sp., L. external mold—dorsal view, M. peel. Scale bar = 1 cm.
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Phylum Annelida

Small mineralized tube fragments of polychaete worms (e.g., UF-I 263086) were collected from Alajuela Formation sediments at locality 630041 and occasionally found attached to calcitic skeletal remains of other invertebrates, mostly mollusks, from other sites.

Phylum Mollusca

Four classes of mollusks (Bivalvia, Scaphopoda, Gastropoda, and Cephalopoda) are represented in the 218 specimen lots collected from seven Alajuela Formation localities (630041, 630043, 630044, 630048, 630049, 630053, and 630061). Of these, nearly 75% are bivalves, and approximately 25% are gastropods. Additionally, one scaphopod and one cephalopod taxon were recovered (see Table 2).
Interestingly, although much more abundant in terms of numbers of specimens, the bivalves are represented by only 13 families while the less abundant gastropods are represented by 19 families (most families based on just one or a few specimens). Common bivalve families represented in the Alajuela deposits are typically infaunal and include the Carditidae, Cardiidae, Veneridae, Corbulidae, and Hiattellidae (i.e., genus Panopea). Most are suspension feeding burrowers that inhabit shallow marine or estuarine environments. Common epifaunal families include Flemingostreidae (i.e., genus Crassostrea) and Pectinidae. At locality 630041, a large, well-developed Crassostrea oyster bar is preserved in situ. These Crassostrea valves are typically very large (up to 24 cm) and moderately to highly eroded. Some specimens resemble modern Crassostrea gigas (Giant Pacific Oyster). Additionally, at locality 630053 only calcitic body fossils of the bivalve families Plicatulidae (i.e., genus Plicatula) and Pectinidae (e.g., genera Euvola and Nodipecten) were recovered; no moldic material was found.

A single, recrystallized scaphopod (tusk shell) was recovered from locality 630044. Scaphopods are infaunal predators.

By far the most common gastropod family from the Alajuela deposits is Calyptraeidae (i.e., genus Calyptraea). Calyptraea, recovered from localities 630043, 630044, 630048 and 630050, today inhabits near-shore, shallow marine environments. Numerous other gastropod families (18) are represented in localities of the Alajuela Formation mentioned above (see Table 2). However, most are very rare.

From picked sediments of locality 630041, two guard-like sheaths (the calcareous covering that envelopes all or part of the phragmocone) of coleoid cephalopods were also discovered (UF-I 264446 and UF-I 263088). This is the first report of fossil coleoids from the Cenozoic of Panama.

**Phylum Arthropoda**

Acorn barnacle (Family Balanidae) shells and isolated opercular plates from at least two genera were observed in many of the Alajuela Formation outcrops along the lake. Collections from six localities (630041, 630043, 630044, 630048, 630049, and 630053) yielded 39 lots of material and complete barnacle shells measured up to 6.5 cm in height. No opercular plates (terga or scuta) were visible inside any barnacle shells and the isolated opercular plates were mostly small. Both shells and opercular plates exhibit high degrees of abrasion. Additionally, some of the larger barnacles have small calcite crystals between the inner and outer walls of their abraded shells. Further preparation of matrix-filled shells may yield associated opercular plates which will allow for confident identification. Tentatively, the larger barnacles are placed in the genus Tamiosoma. Most suspension-feeding acorn barnacles inhabit shallow water environments attached to hard substrates (e.g., rocks, mollusk shells). Other common arthropod fossils found in the Alajuela Formation include several species of the infaunal, deposit-feeding mud shrimp (Family Callianassidae). Remains include isolated fingers and rarer propodi picked from sediments collected at localities 630041, 630043, 630048, and 630049. As the common name suggests, callianassid shrimp typically inhabit burrows in muddy to sandy bottom intertidal to occasionally subtidal marine habitats.

**Phylum Echinodermata.** Remains of fossil echinoderms are rare in the Alajuela Formation exposed on the shores of Lago Alajuela and mostly consist of fragmented radioles (spines) from one (or more?) species of irregular (infaunal) echinoid. All were picked from sediments derived at two localities, 630048 and 630053. However, three regular (epifaunal) echinoid radioles (UF-I 261119) were also collected from locality 630053 and are tentatively identified as belonging to the genus Prionocidaris. Additionally two echinoid tests, one a small (< 1 cm), poorly preserved, unidentifiable sand dollar (UF-I 261085) from locality
630043 and one a large (13.5 cm), exceptionally preserved test of the genus Clypeaster (UF-I 261132) from locality 630061 were collected. The Clypeaster closely resembles Clypeaster gatuni Jackson, 1917, from the Gatun Formation, in shape (length and width) but the Alajuela Formation specimen is much more inflated. Today, Clypeaster occurs in shallow-water, tropical marine environments.

Vertebrate Fauna

Chondrichthyes

So far, 42 chondrichthyan teeth have been collected from the shorelines and islands of Lago Alajuela, representing five different families: Otodontidae, Carcharhinidae, Sphyrnidae, Pristiidae, and Myliobatidae (see representative examples in Fig 9). By no means is this a comprehensive collection of all taxa likely present; however, the relative abundances of the specimens known thus far are as follows: The family Otodontidae is represented by a single species, Carcharocles megalodon. It is the most abundant taxon (N = 15) and likely reflects collection bias towards larger, more visible fossils seen during surface prospecting. Carcharhinidae is the most speciose family, consisting of Carcharhinus spp. (N = 7), Negaprion brevirostris (N = 6), and Hemipristis serra (N = 2). Sphyrnidae is represented by a single specimen attributed to Sphyrna mokarran based on its moderate size, deep distal notch, and serrated cutting edges. The family Myliobatidae is represented by 10 fragmentary teeth, most of which can likely be attributed to Myliobatis sp. or Rhinoptera sp. Hovestadt and Hovestadt-Euler [43] proposed that intergeneric differences within Myliobatinae, in particular the locking mechanism between teeth, enable distinction between genera; however interspecific variability within genera, such as Myliobatis, may be too great to warrant species-level identifications. Similarly, Cappetta [44] noted significant variations between species within the genus Rhinoptera. Consequently, species-level identifications were not justified for teeth attributed to Myliobatidae; and given the fragmentary preservation of the teeth, generic assignments may require more rigorous comparisons with other fossil and living representatives.

Among the eight taxa recognized, C. megalodon and H. serra are extinct, but both have a cosmopolitan distribution in the fossil record [2]. Hemipristis serra is especially common in neritic, warm-water environments during the Miocene and Pliocene [44]. Negaprion brevirostris and Sphyrna mokarran are extant taxa with a circumtropical distribution [45]. Both taxa are often associated with reef environments and generally populate waters less than 100 m depth [45, 46]. Carcharhinus spp. are the most abundant sharks in modern nearshore environments [47, 48] and occur in all temperate and tropical seas [44]. A single rostral denticle was collected by John Turner in 1959. Its flattened conical shape is diagnostic of the sawfish Pristis sp., which is a genus with a widespread fossil record during the Cenozoic [7]. Pristis sp. also has a cosmopolitan distribution today and inhabits marine tropical and subtropical waters, but may enter freshwater and estuarine environments as well [49, 50]. Within the Neotropics, Pristis sp. has been documented from the fossil record of Venezuela, Costa Rica, and Panama [51]. Genera within the family Myliobatidae are often found in tropical to warm temperate seas, especially in shallow waters [43]. All taxa indicate a tropical, shallow water environment; however additional collecting must be undertaken in the future to gain a more holistic perspective of the chondrichthyan fauna of Lago Alajuela.

Testudines

Turtle shell fragments are frequently encountered in outcrops of the Alajuela Formation, although many of them are difficult to identify below the family level because of the lack of diagnostic characters in the preserved material. These fossils, which were recently reviewed by
Fig 9. Representative examples of vertebrate fossils collected from the Alajuela Formation, Lago Alajuela, Panama. Most of these fossils were collected from Interval 1, within a zone bracketed by the Sr-ratio age determination (Fig 3). A. UF-V 318900, *Carcharocles megalodon*, right upper anterior tooth, lingual view. B. UF-V 319140, *Hemipristis serra*, right upper tooth, lingual view. C. UF-V 319139, *Sphyra mokarran*, tooth, lingual view. D. UF-V 318917, *Carcharhinus* sp., right upper tooth, lingual view. E. UF-V 318927, *Negaprion brevirostris*, lower tooth, lingual view. F. UF-V 319131, *Myllobatidae*, partial tooth, ventral view. G. UF-V 318943, *Diodontidae*, right and left fused dentaries, dorsal view. H. UF-V 318945, *Crocodylia*, tooth, anterior or posterior view. I. UF-V 318949, *Crocodylia*, partial osteoderm, dorsal view. J. UF-V 318954, *Aves*, distal left humerus, medial (top) and lateral (bottom) views. K. UF-V 318953, *Gomphotherium* sp., tooth fragment. L. UF-V 403570, *Gomphotherium* sp., anterior portion of left dp2, occlusal (top) and buccal (bottom) views. M. UF-V 318955, *Tayassuidae*, right astragalus, anterior view. N. UF-V 403571, *Carnivora*, left I3, distal (left) and mesial (right) views. O. UF-V 321263, *Dinohippus* sp., right upper P2, buccal (top) and occlusal...
Bourque et al. [52], consist of at least four taxa (Table 3). In the exception to the rule in terms of fossilization, Bourque et al. [52] describe a well-preserved partial skull of a side-necked turtle that they refer to a new species of the pleurodire Bairdemys. The reminder of the testudine samples are represented by cryptodires, including soft-shelled turtles (Trionychidae), tortoises (Testudinidae), and sea turtles (Chelonia). Although not a taxonomically diagnostic collection, the paleoecological implications for the presence of these four testudinid groups will be described below.

Crocodylia

Isolated crocodylian elements were collected from several localities along the shores of Lago Alajuela. Crocodilian fossils come from Interval 1, most of them were collected in the fine and medium grained sandstones, whereas others were found in amalgamated conglomerate lenses. Isolated vertebrae have fused neurocentral sutures [53] and represent mature individuals. Their size is comparable to that of living crocodylians between 3–4 m long. Most osteoderms, although incomplete, preserve straight margins and well defined corners, indicating that their shape was roughly square. They also lack keels on their external surfaces. Crocodilian teeth are represented by two morphotypes, i.e., a slender, sharp morphotype, and blunt, robust one.

The most diagnostic elements recovered so far are an almost complete axis and partial maxilla. Both have features consistent with longirostrine crocodylians. However, they lack key features diagnostic of living Gavialis and South American gavialoids. These remains probably represent tomistomines, likely related to the North American genus Thecachampsas (= Gavialosuchus). Crocodylian fossils attributed to Thecachampsas have been collected in the upper Miocene Curri Formations of Costa Rica [54]. Longirostrine crocodylians from the early Miocene of the Panama Canal basin could also be related to Thecachampsas [55].

Aves

A single fragmentary left distal humerus, UF-V 318954 (Fig 9), although identifiable as a bird, is otherwise not taxonomically diagnostic (Steadman, personal communication, 2015).

Mammalia

**Cf. carnivora.** A single left I3, UF-V 403571, is attributable to a carnivoran, but is not diagnostic at a lower taxonomic level.

**Cf. dugongidae.** Several rib fragments have been collected that are otherwise undiagnostic except for likely being from a sirenian based on the oval cross-section and internal pachyostotic condition characteristic of these marine mammals. It is tempting to speculate that these might pertain to the cosmopolitan Miocene genus Metaxytherium, but this is not warranted until more diagnostic material is collected because there are several endemic Caribbean extinct dugongids [56].

**Proboscidea.** The genus Gomphotherium was recently described from Lago Alajuela [6] based on a diagnostic 1st lower molar, UF-V 294322. In July 2015 we confirmed with the collector, John Turner, where the tooth was discovered in the field in 1959; the approximate location (between 630042 to 630049) also pertains to Interval 1 in our composite stratigraphic section (Fig 3). An additional specimen, represented by a left dp2 (UF V 403570), has been recovered from Isla Vigia (Table 1, 630050). Other proboscidean tooth fragments that likely
### Table 3. Preliminary list of fossils collected from the Alajuela Formation, late Miocene of Panama.

| Plants (Angiosperms) |
|----------------------|
| **Arecales**         |
| Arecales (Palm family) |
| *Palmoxylon* sp. A  |
| *Palmoxylon* sp. B  |
| **Fabales**          |
| Fabaceae (Legume family) |
| gen. et sp. indet.   |
| **Magnoliidae**      |
| Annonaceae (Custard apple family) |
| *Annonoxylon* sp.    |
| **Malpighiales**     |
| Chrysobalanaceae     |
| *Parinarioxyloyn* sp.|
| Humiriaceae          |
| *Humiriaceoxylon* sp.|
| ?Phyllanthaceae      |
| gen. et sp. indet.   |
| (cf. Achariaceae, Malpighiaceae) |
| **Malvales**         |
| Malvaceae (Mallow family) |
| gen. et sp. indet.   |
| **Sapindales**       |
| Sapindaceae (Soapberry family) |
| gen. et sp. indet.   |
| **Invertebrata**     |
| **Brachiopoda**      |
| Lingulidae           |
| *Glottidia* sp.      |
| **Bryozoa**          |
| Cheilostomata        |
| Chapperiidae         |
| *Chaperia* sp.       |
| Hippoporidae         |
| Family indet.        |
| *Hippoporidra* sp.   |
| **Cyclostomata**     |
| Plagioeciidae        |
| gen. et sp. indet.   |
| Family indet.        |
| **Annelida**         |
| Polychaeta           |
| Family indet.        |
| **Mollusca**         |
| Bivalvia             |
| Yoldiidae            |
| Arcidae              |
| Pectinidae           |

(Continued)
Table 3. (Continued)

| Family | Genus      |
|--------|------------|
| Euvola | sp.        |
| Nodipecten | sp.      |
| Plicatulidae | Plicatula sp. |
| Anomidae | Anomia sp. |
| Flemingostreidae | Crassostrea sp. |
| Cardiidae | Venericostrea sp. |
| Pharidae | Eris sp. |
| Tellinidae | Veneridae |
| Corbulidae | Panopea sp. |
| Gastrochaenidae | Hiatellidae |
| Scaphopoda | Family indet. |
| Gastropoda | Cerithiidae |
| | Turritellidae |
| | Strombidae |
| | Strombus sp. |
| Xenophoridae | Xenophora sp. |
| Calyptraeidae | Calyptraea sp. |
| Cypraeidae | Malea sp. |
| Naticidae | Cymatophos sp. |
| Tonnidae | Melongenidae |
| | Melongena sp. |
| Buccinidae | Turbinellidae |
| | Turbinella sp. |
| Olivellidae | Olivella sp. |
| Olividae | Oliva sp. |
| Table 3. (Continued) |
|-----------------------|

| Marginellidae          | Conidae |
|------------------------|---------|
| Conus sp.              |         |
| Terebriidae            | Terebra sp. |
| Turridae               |         |
| Architectonicae        |         |
| Haminoeidae            |         |
| Cephalopoda            | Coleoidea |

**Arthropoda**

| Malacostraca           | Callianassida |
|------------------------|---------------|
| gen. et sp. undet.     |               |
| Cirripedia             | Balanidae     |
|                       | cf. Tamiosoma sp. |

**Echinodermata**

| Echinoidea             | Cidaridae     |
|------------------------|---------------|
|                       | cf. Prinocidaris sp. |
| Clypeasteridae         | Clypeaster sp.  |

**Vertebrata**

| Chondrichthyes         | Lamniformes   |
|------------------------|---------------|
| Otodontidae            | Carcharocles megalodon |
| Carcharhiniformes      | Carcharhinidae |
|                       | Hemipristis serra |
|                       | Carcharhinus spp. |
|                       | Negaprion brevirostris |
|                       | Sphymidae      |
|                       | Sphyrna mokarran |
| Pristiformes           | Pristidae     |
|                       | Pristis sp.    |
| Myliobatiformes        | Myliobatidae  |
|                       | Myliobatis sp. |
|                       | Rhinoptera sp. |

**Reptilia**

| Testudines*            | Podocnemididae |
|------------------------|-----------------|
|                       | Bairdemys n. sp. |
|                       | Trionychidae    |
|                       | gen. et sp. indet. |

(Continued)
pertain to this genus have been collected (Fig 9) from several of our localities, but primarily from the lower part of the section (Interval 1). *Gomphotherium* has a relatively long biochronological range in North America from about 15 to 5 Ma. In the absence of other chronological information and the lack of a definitive species determination for this genus, we were unable to make a more precise age determination for the Alajuela Formation. But, with the new Sr-ratio age determinations reported here, the occurrence of *Gomphotherium* in Panama has significance with regard to previous hypotheses concerning the timing of dispersal into Central America during the Miocene.

*Cf. tayassuidae.* A single right astragalus, UF-V 318955 (Fig 9), indicates the presence of a medium-sized artiodactyl that likely represents a tayassuid based on our comparisons with other specimens of Miocene peccaries in the UF-V collection.

**Equidae.** Two specimens of equids have been collected, including (1) a right P2 (UF-V 321263) of *cf. Dinohippus* sp. (Fig 9), likely referable to this genus based on tooth size, simple enamel pattern, and the strength of the connection of the protocone to the protoselene; and (2) a partial upper molar fragment (UF-V 321264) likely referable to *Cormohipparion* sp. based on tooth size and the complexity of enamel plications.

### Discussion and Significance

#### Biochronology and Age Assignment

All of the taxa collected from Lago Alajuela so far have only been identified to the genus or higher taxonomic level, with the exception of some of the species of sharks. We anticipate that as more fossils are collected of temporally diagnostic taxa and additional detailed studies of

| Table 3. (*Continued*) |
|-------------------------|
| Testudinidae             | gen. et sp. indet. |
| Cheloniidae             | gen. et sp. indet. |
| Crocodylia (longirostrine) | Tomistominae       |
| Aves                     | gen. et sp. indet. |
| Mammalia                 |                     |
| cf. Carnivora            |                     |
| Sirenia                  |                     |
| cf. Dugongidae           |                     |
| Proboscidea              |                     |
| Gomphotheriidae          |                     |
| Gomphotherium sp.        |                     |
| Artiodactyla             |                     |
| Tayassuidae              | gen. et sp. indet.  |
| Perissodactyla           |                     |
| Equidae                  |                     |
| Cormohipparion sp.       |                     |
| Dinohippus sp.           |                     |

*From Bourque et al. [52].

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individual groups are undertaken in the future, some of the fossils collected from Lago Alajuela will be identified to species. As such, these fossils may then provide a more precise biochronological assessment of the Alajuela Formation.

The invertebrate taxa represent genera that are long-lived and are known to have occurred during the Neogene. Likewise, the fossil plants found adjacent to Lago Alajuela are referred to modern families. With regard to the vertebrates, the sharks and reptiles likewise have long temporal ranges, and as such, are not particularly useful biochronologically, other than also being late Cenozoic or Neogene, similar to the invertebrates. With regard to the mammals, the proboscidean *Gomphotherium* is known to have occurred elsewhere in North America during the middle to late Miocene and early Pliocene, from ca. 15 to 5 Ma [6]. Despite its relatively long range in higher-latitude North America, Lucas and Alvarado [57] assert that *Gomphotherium* did not disperse into lower latitudes, i.e., Central America, until the late Hemphillian. The two equid genera have late Miocene to early Pliocene temporal ranges, and are similar to those forms previously described from Costa Rica [58] that are referred to the Hemphillian North American Land Mammal Age, which spans an interval from 9 to 4.8 Ma in higher-latitude North America [59].

Given the current state of our knowledge about the fossil faunas and floras from Lago Alajuela, the biochronological information suggests a late Miocene age for the Alajuela Formation. We are hopeful that we might find diagnostic and biogeographically interesting microfossils, e.g., foraminifera and mammals such as rodents, bats, or possibly even primates (e.g., Bloch et al. [60]) through screen-washing and sieving, but up to now we have been unsuccessful in this regard. As we continue to process and sort screenwashed matrix, the potential for adding to the vertebrate fauna remains a distinct possibility.

As such, we must turn to the Sr-ratio ages derived from *in situ* invertebrate fossils to provide additional clarity in terms of the age of the Alajuela Formation. It should be noted here that most of the diagnostic mammal taxa are collected from the same zone as the Sr-ratio ages (Fig 3). The mean age of 9.77 ± 0.22 Ma (Table 1) places our measured section within the late Miocene, and latest Clarendonian, not Hemphillian, NALMA (Cl3, sensu [59]). A close temporal equivalent in higher latitude North America is represented by the Love Bone Bed Local Fauna of north-central Florida [61].

**Paleoecology**

Taken together, the plant, invertebrate, and vertebrate fossils from the Alajuela Formation are diagnostic in terms of reconstructing the paleoenvironments and paleoecology during the late Miocene in this region of what is now Panama. The sharks, sirenians, and some of the invertebrates (e.g., *Clypeaster*) indicate a shallow-marine environment. The land mammals, other invertebrates (e.g., *Crassostrea*), and ichnofossils indicate an estuarine environment with local influence from terrestrial environments. Terrestrial vertebrate fossils include the proboscidean, horses, peccary, carnivoran, and tortoise. The trionychid turtle indicates freshwater habitats. The crocodilians and some of the other turtles confirm the presence of transitional estuarine and shallow marine environments.

We understand that the silicified woods in the Alajuela Formation are likely reworked, and are unsure how much older these fossils are relative to the in situ invertebrates and vertebrates. Regardless, all of these wood families are common in tropical forests in Panama today [21, 22], and from an analysis of their anatomy indicate the presence of large trees. This evidence thus speaks to a temporal duration from the early Miocene to the Recent in which these habitat-types are represented in Panama.
Late Miocene Seaway in Panama

Although traditionally it has been argued that the Central America Seaway functioned until the late Miocene, with final closure during the Pliocene at about 4 to 5 Ma, recent evidence argues for an earlier closure. Montes et al. [40] suggest that closure of the seaway occurred significantly earlier, i.e., during the middle Miocene, ca. 13 to 15 Ma. The younger evidence from Alajuela as well as from roughly contemporaneous localities from the Gatun [1] and Chucunaque [62] formations in Panama speak to this debate.

The abundance of marine fossils from the Alajuela and Gatun formations, and as shown by Coates et al. [5] for the Chucunaque Formation, demonstrates seaway connections existed through central Panama during the late Miocene. Strontium ratios also suggest the Alajuela Formation overlaps in time with the richly fossiliferous upper Miocene Gatun Formation to the north with which it shares many invertebrate faunal elements. Preliminary interpretations suggest the Alajuela Formation represents a higher energy, near-shore, marine setting that episodically received riverine/terrestrial input and was perhaps shallower and more proximal to a coastline than either the Chucunaque or Gatun formations. Future work will help to elucidate these important relationships.

Concluding Comments

The discovery of a new fossil assemblage of plant, invertebrate, and vertebrate fossils from the Alajuela Formation at Lago Alajuela has the potential to advance our understanding of the evolution of tropical marginal marine and terrestrial ecosystems in Central America, as well as further address the current debate about the formation of the isthmus. The calibration of this assemblage using Sr-isotope ratio dating to 9.77 Ma ± 0.22 Ma provides precise temporal control otherwise unknown for the late Miocene in Central America. This indicates contemporaneity with the Gatun and Chucunaque formations in Panama as well as a late Clarendonian (Cl3) North American Land Mammal age as this biochron is otherwise known in higher latitude North America. We understand that we have not yet sampled the full range of ancient diversity at Lago Alajuela; we hope that further discoveries will be forthcoming with additional field work. Likewise, the taxonomic assignments that we present here will likely become more refined as individual taxonomic groups are compared in more detail in the future.

Supporting Information

S1 Table. Analysis and comparisons of fossil woods studied.
(XLSX)

S2 Table. Panama REE (rare earth element) data analyzed in? 2015.
(XLSX)

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Author Contributions

Conceptualization: BJM DSJ NAJ JWM GSM RVP VJP SMM ARW.

Data curation: RWP NAJ VJP SMM.

Funding acquisition: BJM DSJ GSM.

Investigation: BJM DSJ NAJ JWM GSM RVP VJP SMM ARW.

Project administration: BJM.

Supervision: BJM.

Visualization: BJM DSJ NAJ ARW RWP SMM.

Writing – original draft: BJM DSJ NAJ JWM GSM RVP VJP SMM ARW.

Writing – review & editing: BJM DSJ NAJ JWM GSM RVP VJP SMM ARW.

References

1. Hendy AJW. Spatial and stratigraphic variation of marine paleoenvironments in the middle-upper Miocene Gatun Formation, Isthmus of Panama. Palaios. 2013; 28:210–227.

2. Pimiento C, Gonzalez-Barba G, Ehret DJ, Hendy AJW, MacFadden BJ, Jaramillo C. Sharks and rays (Chondrichthyes, Elasmobranchii) from the late Miocene Gatun Formation of Panama. J. Paleontol. 2013; 87:755–774.

3. MacFadden BJ, Bloch JI, Evans H, Foster DA, Morgan GS, Rincon AF, Wood AR. Temporal calibration and biochronology of the Centenario Fauna, early Miocene of Panama. J Geol 2014; 122:113–135.

4. Lucas SG. Late Pleistocene mammals from El Hatillo, Panama; Mamíferos del Pleistoceno Terminal de la localidad de El Hatillo, Panamá. Revista Geol Amér Central. 2014; 50:139–151.

5. Coates AG, Collins LS, Aubry MP, and Berggren WA. The geology of the Darien, Panama, and the late Miocene-Pliocene collision of the Panama arc with northwestern South America. Bull Geol Soc Am 2004; 116:1327–1344.

6. MacFadden BJ, Morgan GS, Jones DS, Rincon AF. 2015. Gomphotherium proboscidean (Gomphotherium) from the late Neogene of Panama. J Paleont. 2015; 89:360–365.

7. PBDB (Paleobiology Database) [Internet]. [cited 25 July 2015]. https://paleobiodb.org.

8. Coates AG, Obando JA. The geologic evolution of the Central American isthmus. In: Jackson JBC, Budd AF, Coates AG, Editors. Evolution and environment in tropical America. Chicago: Univ. Chicago Press; 1996. p. 21–56.

9. Morton ES. Intratropical migration in the Yellow-Green Vireo and Piratic Flycatcher. The Auk 1977; 94:97–106.

10. Woodring WP Geology and paleontology of Canal Zone and adjoining parts of Panama. Geological Survey Professional Paper 306-A. U S Gov Print Office, Washington DC 1957. p. 1–239.

11. Woodring W, Stewart R, Stewart J Geologic map of the Panama Canal. Reston [VA]: US Geol Surv. 1980.

12. Kirby MX, Jones DS, MacFadden BJ. Lower Miocene stratigraphy along the Panama Canal and its bearing on the Central American peninsula. PLoS ONE. 2008; 3(7):e279.

13. McArthur JM, Howarth RJ, Bailey TR. Strontium isotope stratigraphy: LOWESS version 3: Best fit to the marine Sr-isotope curve for 0–509 Ma and accompanying look-up table for deriving numerical age. J Geol. 2001; 109:155–170.

14. Haas H, Rowe NP. Thin sections and wafering. In: Jones TP, Rowe NP, editors. Fossil plants and spores: Modern techniques. London: Geol Soc.1999 p. 76–81.

15. InsideWood. 2004-onwards. Published on the Internet. http://insidewood.lib.ncsu.edu/search.

16. Wheeler EA. Inside Wood—A web resource for hardwood anatomy. IAWA Journal. 2011; 32:199–211.

17. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons, Vols. 1 & 2. Oxford: Clarendon Press; 1950.

18. Henderek RL, Moreno-Bernal JW, Von Dassow W, Wood AR, Hendy AJ, Whiting E, et al October. Geologic reconnaissance of the middle-late Miocene Alhajuela [sic.] Formation (Panama): Implications for
shoaling of the Central American Seaway. 2014. Geol Soc Am Annual Meeting in Vancouver, British Columbia. Paper 56–8.

19. Dalrymple RW, Zaitlin BA, Boyd R Estuarine facies models: conceptual basis and stratigraphic implications: perspective. J Sed Res 1992; 62:1130–1146.

20. Pollard JE, Goldring R, Buck SG. Ichnofabrics containing Ophiomorpha: significance in shallow-water facies interpretation. J. Geol. Soc. 1993; 150:149–164.

21. Burnham RJ, Johnson KR. South American palaeobotany and the origins of Neotropical Rainforests. Phil Trans Royal Soc B: Biol Sci. 2014; 359:1595–1610.

22. Gentry AH Neotropical floristic diversity: Phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean Orogeny? Annals Missouri Bot Gard 1982; 69:557–593.

23. Wheeler EA, Baas P, Rodgers S. Variations in dicot wood anatomy: a global analysis based on the Insidewood Database. IAWA Journal. 2007; 28:229–258.

24. Fichtler E., Worbes M. Wood anatomical variables in tropical trees and their relation to site conditions and individual tree morphology. IAWA Journal 2012; 33:119–140.

25. Olson ME, Anfodillo T, Rosell JA, Petit G, Criveller A, Issard S, et al. Universal hydraulics of the flowering plants: vessel diameter scales with stem length across angiosperm lineages, habits and climates. Ecol Lett. 2014; 17:988–997. doi: 10.1111/ele.12302 PMID: 24847972

26. Dickison WC. Steps towards the natural system of the Dicotyledons: Vegetative anatomy. Aliso 1989; 12:555–566.

27. Wheeler EA, Baas PA. Survey of the fossil record for dicotyledonous wood and its significance for evolutionary and ecological wood anatomy. IAWA Journal 1991; 12:275–318.

28. Thomas R, Boura A. Palm stem anatomy: phylogenetic or climatic signal? Bot J Linnean Soc. 2015; 178:467–488.

29. MacFadden BJ, Symister C, Cannarozzi N, Pimiento C, DeGracia C. Comparative diagenesis and Rare Earth Element (REE) variation in Miocene invertebrate and vertebrate fossils from Panama. J Geol. 2015; 123:491–507.

30. Rodríguez-Reyes O, Falcon-Lang H, Gasson P, Collinson M, Jaramillo CA. Fossil woods (Malvaceae) from the lower Miocene (early to mid-Burdigalian) part of the Cucaracha Formation of Panama (Central America) and their biogeographic implications. Rev. Palaeobot. Palynol. 2010; 209:11–34.

31. Jud NA, Nelson CW, Herrera F. Fruits and wood of Parinari from the early Miocene of Panama and the fossil record of Chrysobalanaceae. Amer J Bot 2016; 103:277–289.

32. Trueman CN, Benton MJ. A geochemical method to trace the taphonomic history of reworked bones in sedimentary settings. Geology 1997; 25:263–266.

33. Herrera F, Manchester SR, Jaramillo CA, MacFadden BJ, da Silva-Caminha SA. Phytogeographic history and phylogeny of the Humiriaceae. Int J Plant Sci 2010; 171:392–408.

34. Herrera F, Manchester SR, Koll R, Jaramillo CA. Fruits of Oreomunnea (Juglandaceae) in the early Miocene of Panama. In: Stevens WD, Montiel OM, and Raven PH, editors. Paleobotany and Biogeography: A Festschrift for Alan Graham in His 80th Year. St. Louis: Missouri Bot Gard Press. 2014, p. 124–33.

35. Graham A. Studies in Neotropical Paleobotany. IV. The Eocene communities of Panama. Annals Missouri Bot Gard 1985; 72:504–534.

36. Graham A. Studies in Neotropical Paleobotany. VI. The lower Miocene communities of Panama-the Cucaracha Formation. Annals Missouri Bot Gard 1986; 75:1467–79.

37. Graham A. Studies in Neotropical Paleobotany. VII. The lower Miocene communities of Panama-the La Boca Formation. Annals Missouri Bot Gard 1989; 76:50–66.

38. Jaramillo C, Moreno C, Ramirez D, Silva S, de la Barrera A, da Silva S, de la Barrera A. In: Stevens WD, Montiel OM, and Raven PH, editors. Paleobotany and Biogeography: A Festschrift for Alan Graham in His 80th Year. St. Louis: Missouri Bot Gard Press. 2014, p. 124–133.

39. Montes C., Cardona A., Jaramillo C., Pardo A., Silva J. C., Valencia V., et al. Middle Miocene closure of the Central American Seaway. Science 2015; 348:226–229. doi: 10.1126/science.aaa2815 PMID: 25859042

40. Campbell MR, Campbell DC, Campbell LD, Portell RW. Distribution and paleoecology of Glottidia inexpectans Olsson, 1914 (Brachiopoda: Lingulidae). Tulane Studies Geol Paleontol. 1997; 30:159–170.

41. Emig CC. Ecology of inarticulated brachiopods. In:Williams A, Brunton CHC, Carlson SJ, editors. Brachiopoda. Treatise on Invertebrate Paleontology (revised), Univ Kansas and Geol Soc Amer; 1997. Part H, p. 473–495.
43. Hovestadt DC, Hovestadt-Euler M. Generic assessment and reallocation of Cenozoic Myliobatinae based on new information of tooth, tooth plate and caudal spine morphology of extant taxa. Palaeontos. 2013; 24: 1–66.

44. Cappetta H. Chondrichthyes II: Mesozoic and Cenozoic Elasmobranchii. G. Fischer Verlag, Stuttgart; New York. 1987.

45. Compagno L.J.V. FAO Species Catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. FAO Fisheries Synopsis. 1984. p. 1–655.

46. Compagno L.J.V. Dando M., and Fowler S. Sharks of the world. 2005. p. 1–368.

47. Kent BW. Fossil sharks of the Chesapeake Bay Region. Columbia, Maryland: Egan Rees and Boyer, Inc., 1994 146 p.

48. Naylor GJP, Marcus LF. Identifying isolated shark teeth of the genus Carcharhinus to species: relevance for tracking phylogenetic change through the fossil record. Amer Mus Novitats. 1994; 3109:1–53.

49. Séré B. Pristidae. In: Paugy D, Lévêque C, Teugels GG, editors. The fresh and brackish water fishes of West Africa Volume 1. Collection Faune et Flore Tropicales 40. Mus nat d’hist [Paris, France] and Mus royal Afrique Central [Tervuren, Belgium]. 2003. p. 78–80.

50. Carvalho, MR, Séré B, Schelly RC. Pristidae. In: Stiasny MLJ, Teugels GG, Hopkins CD, editors. The fresh and brackish water fishes of Lower Guinea, West-Central Africa. Volume I. Collection Faune et Flore tropicales 42. Museum National d’Histoire Naturelle: Paris, France, and Musée Royal de l’Afrique Centrale: Tervuren, Belgium; 2007. p. 148–153.

51. Aquilera OA, Ramos MIF, Paes ET, Costa SARF, Sánchez-Villagra MR. The Neogene tropical America fish assemblage and the paleobiogeography of the Caribbean region. Swiss J Paleontol. 2011; 130:217–240.

52. Bourque JR, Wood AR, Hendy AJW. Turtles from the late Miocene of central Panama. 2016 Forthcoming.

53. Brochu CA. Closure of neurocentral sutures during crocodilian ontogeny: Implications for maturity assessment in fossil archosaurs. J Vert Paleontol. 1996; 16:49–62.

54. Laurito CA, Valerio AL. The first record of Gavialosuchus americanus Sellards. 1915. †(Eusuchia: Crocodylidae, Tomistominae) for the late Tertiary of Costa Rica and Central America. Revista Geol Amér Central. 2008; 39:107–115.

55. Hastings AK, Bloch JI, Jaramillo CA, Rincon AF, MacFadden BJ. Systematics and biogeography of crocodylians from the Miocene of Panama. J Vert Paleontol. 2013; 33:239–263.

56. Domning DP. Sireniens, seagrasses, and Cenozoic ecological change in the Caribbean. Palaeoecogr Palaeoclimatol Palaeoecol 2001; 166:27–50.

57. Lucas SG, Alvarado GE. Fossil Proboscidea from the Upper Cenozoic of Central America: Taxonomy, evolutionary and paleobiogeographic significance. Revista Geol Amér Central. 2011; 42:94–42

58. Laurito CA, Valerio Z AL. Los caballos fósiles de la formación Curré Cantón de Coto Brus, Costa Rica. San José, Mus Nac Costa Rica. 2010;131 p.

59. Tedford RH, Albright LB III, Barnosky AD, Ferrusquia-Villafranca I, Hunt RM Jr, Storer JE, et al. Mammalian biochronology of the Arkamekan through Hemphillian interval (late Oligocene through early Pliocene epochs). In: Woodburne MO, editor. Late Cretaceous and Cenozoic Mammals of North America: Biostratigraphy and Geochronology. New York: Columbia University Press; 2004. p. 169–231.

60. Bloch JI, Woodruﬀ ED, Wood AR, Rincon AF, Harrington AR, Morgan GS, Foster DA, Montes C, Jaramillo CA, Jud NA, Jones DS, and MacFadden BJ. First North American fossil monkey and early Miocene tropical biotic interchange. Nature 2016;

61. Webb SD, MacFadden BJ, Baskin JA. Geology and paleontology of the Love Bone Bed from the late Miocene of Florida. Amer J Sci 1981; 281:513–544.

62. Perez V, Pimiento C, Hendy A, González-Barba G, Hubbell G, MacFadden BJ. Late Miocene chondrichthysans from Lago Bayano, Panama: Functional diversity, environment, and biogeography. J Paleontol. Forthcoming 2016.