Fossil berries reveal global radiation of the nightshade family by the early Cenozoic

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Summary

- Fossil discoveries can transform our understanding of plant diversification over time and space. Recently described fossils in many plant families have pushed their known records farther back in time, pointing to alternative scenarios for their origin and spread.
- Here, we describe two new Eocene fossil berries of the nightshade family (Solanaceae) from the Esmeraldas Formation in Colombia and the Green River Formation in Colorado (USA). The placement of the fossils was assessed using clustering and parsimony analyses based on 10 discrete and five continuous characters, which were also scored in 291 extant taxa.
- The Colombian fossil grouped with members of the tomatillo subtribe, and the Coloradan fossil aligned with the chili pepper tribe. Along with two previously reported early Eocene fossils from the tomatillo genus, these findings indicate that Solanaceae were distributed at least from southern South America to northwestern North America by the early Eocene.
- Together with two other recently discovered Eocene berries, these fossils demonstrate that the diverse berry clade and, in turn, the entire nightshade family, is much older and was much more widespread in the past than previously thought.

Introduction

Our understanding of the history of plant diversification fundamentally relies on the fossil record. Fossilized plant remains allow us to track shifts in vegetation across geological timescales (Palazzesi & Barreda, 2012; Falcon-Lang *et al.*, 2015), identify the origins of novel traits (Crepet & Feldman, 1991; Sun *et al.*, 1998; Friis *et al.*, 2015), trace the history of biotic interactions (Currano *et al.*, 2008), and estimate the timeline of evolutionary events (Benton *et al.*, 2000; Crepet *et al.*, 2004). Even as methods for making inferences about evolutionary history from extinct and extant plant diversity have become more biologically realistic and statically robust (e.g. Slater *et al.*, 2012; Heath *et al.*, 2014), progress continues to be limited by the availability of fossil

material with sufficient preservation to allow precise taxonomic placement. Accordingly, discoveries of new plant fossils have often illuminated previously disputed timelines (Strother & Foster, 2021; Cui *et al.*, 2022) and in some cases overturned widely accepted notions about the timing of plant evolution (Bonacorsi *et al.*, 2020).

While fossils informative about early land and seed plant history have received a great deal of attention, recent fossil discoveries belonging to many extant families and genera have also led to significant transformations in understanding plant diversification in the Cenozoic. For example, the large Melastomatoid clade, once hypothesized to have Laurasian origins in the early Eocene (Renner *et al.*, 2001), is now known to date back to the middle to late Paleocene of Colombia (Carvalho *et al.*, 2021),

suggesting the lineage may instead be Gondwanan. Similarly, discoveries of spectacular Asteraceae pollen and flowers from the Late Cretaceous of West Antarctica and middle Eocene of Argentina, respectively (Barreda *et al.*, 2010, 2015), have pushed crown ages of the family to 90 Ma and implicated a Gondwanan origin for the now cosmopolitan family (Mandel *et al.*, 2019). Despite these exciting findings, many presently widespread and diverse families are characterized by scant fossil records, precluding downstream studies of biogeography and diversification (Stuls & Axsmith, 2011; Kessous *et al.*, 2021).

Here, we investigate the fruit-fossil history of the nightshade family, Solanaceae, a large, cosmopolitan and economically important clade with a paucity of well-preserved fossil information. This family comprises *c.* 2750 species, roughly 64% of which are native to South America (Dupin *et al.*, 2017). Its major lineages (subfamilies and tribes) are all estimated to have originated in South America, with subsequent dispersals over land and water leading to its global distribution (Dupin *et al.*, 2017). Nevertheless, understanding of the timing of diversification for Solanaceae has been hindered by the sparse and fragmentary fossil record, which was further confused by many misidentified specimens (Millan & Crepet, 2014; Särkinen *et al.*, 2018). The first divergence-time estimates, calibrated with the few reliable seed fossils, placed the stem age of the family between 46 and 54 Ma (Särkinen *et al.*, 2013, 2018). However, the subsequent discovery of two species of early Eocene (52 Ma) lantern-fruit fossils (genus *Physalis* L.) from caldera-lake beds in Patagonian Argentina indicated that the family has a deeper history in South America, when the continent was still part of Gondwana, and could have originated in West Gondwana (Wilf *et al.*, 2017; Deanna *et al.*, 2020). The occurrence of fossils from the tomatillo clade with derived features, such as an inflated calyx (Deanna *et al.*, 2019), by 52 Ma also suggested that the family Solanaceae was well diversified by that time (Wilf *et al.*, 2017).

This study examines a broader set of Solanaceae fruit fossils, including the first records from tropical South America (Colombia) and North America (Colorado; Fig. 1). The taxonomic affinities of these fossils span additional lineages from the berry clade (Solanoideae, Särkinen *et al.*, 2013) and are all confidently dated as Eocene (56–33.9 Ma). We consider the implications of the new fossils for the age and biogeographic history of Solanaceae, given the wide distribution of these Eocene berries. In addition to providing insight into the evolution of the nightshade family, these new fossils hint at a previously rich solanaceous flora in northern North America that has now largely disappeared.

Materials and Methods

Geological setting

The fossil material of *Eophysaloides inflata* sp. nov. was collected on the western flank of the Eastern Cordillera in Santander, Colombia, from a fossiliferous locality in lacustrine facies of the Eocene Esmeraldas Formation. The age of the locality was determined based on a biostratigraphic pollen correlation and a chemostratigraphic analysis as middle to late Eocene (Martínez *et al.*, 2021). This site is located in the Nuevo Mundo Syncline,

Middle Magdalena Valley Basin, in the area of the Topocoro Dam near the road that leads from Bucaramanga to Barrancabermeja. The region was first explored in 2013 as part of a paleontological salvage project led by the Smithsonian Tropical Research Institute, the Colombian Geological Survey, and Isagen, a power generation company, during the construction of the dam. The locality has the identification number STRI-430134; it is located at 7°6′30.24″N and 73°25′45.12″W (WGS84), elevation 179 m, and was not flooded after the filling of the dam reservoir. A geological map of the region and the stratigraphic details of the locality can be found in Martínez (2018) and Martínez *et al.* (2021). Approximately 400 specimens were collected from this locality, mostly compressions and impressions of angiosperm leaves, but also including seeds, fruits and flowers (Martínez, 2017, 2018; Pérez-Consuegra *et al.*, 2017; Martínez *et al.*, 2021).

The provenance for all three specimens of *Lycianthoides calycina* sp. nov. is the Parachute Creek Member of the lacustrine Eocene Green River Formation in Colorado, western United States. The stratigraphic framework for the Green River Formation and the Parachute Creek Member, including $^{40}Ar/-^{39}Ar$ ages and other geochronological constraints, can be found in Smith *et al.* (2008) and Smith & Carroll (2015). The holotype, UCM 41276a,b, was collected by David Kohls at the Claudia's Place locality (UCM loc. 2009063; Garfield Co., CO, USA), near the base of the Parachute Creek Member, 39°21′07.5″N, 108°03′17.0″W. The age of the strata is *c.* 51 Ma (Smith *et al.*, 2008). Paratype UCM 41285 was collected at the Anvil Points locality (UCM loc. 2005026; Garfield Co.), *c.* 10–20 m below the Mahogany Zone in the Parachute Creek Member, by David Kohls and Dena Smith, 39°32′08.05″N, 107°56′57.83″W, age *c.* 49.5 Ma (Smith *et al.*, 2008). The second paratype (DMNH EPI.57889a,b) was found by Ron Meyer and collected by Bill Hawes and Kirk Johnson at Ron Meyer's Scorpion locality (DMNH loc. 304; Rio Blanco Co., Vermont, CO, USA) near the base of the Parachute Creek Member, 39°43′47.0″N, 107°58′25.0″W, age *c.* 51.5 Ma (Smith *et al.*, 2008).

Calyx venation patterns

Fossil venation was observed using Zeiss Stemi SV8 and Wild M400 stereomicroscopes. Photography was done with a Nikon D200 camera in Colombia and a Canon XSi 450 with EFS 60 macrolens in the US, with varied low-angle lighting. All the fossil materials from Colombia were studied at the Geosciences Laboratory of the Universidad de los Andes, in Bogotá, Colombia, and all the fossils from the USA were studied at the Florida Museum of Natural History, University of Florida, Gainesville, FL, USA. Calyces were described following the modified version of the standard terminology for leaf architecture (Hickey, 1973; Trivett & Pigg, 1996; Ellis *et al.*, 2009) as adapted for Solanaceae calyces, according to Hamed & Mourad (2004) and D'Arcy (1986).

Morphospace analysis of extant and extinct Solanaceae fruits

We assembled a dataset of 10 discrete and five continuous fruiting characters for seven fossils and 291 extant species of
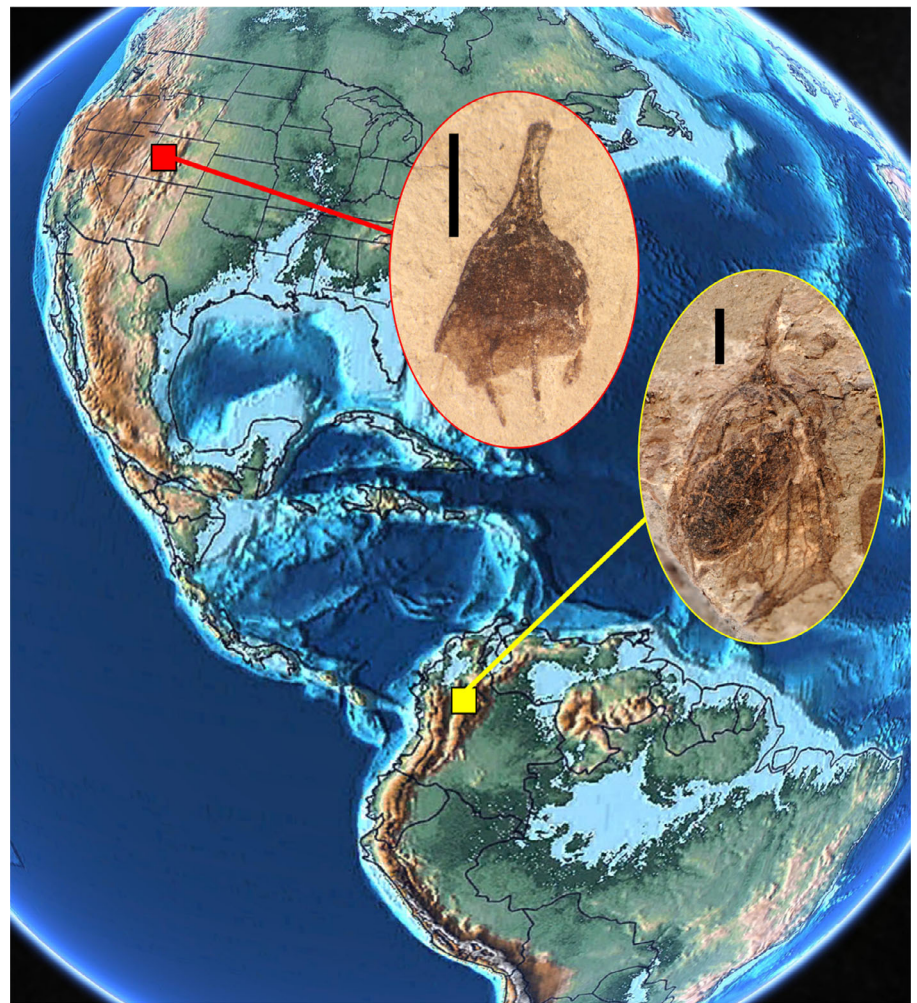
Fig. 1 Middle Eocene paleogeographic reconstruction of North and South America showing the locations of new fossil occurrences. Red square on left, area of the Green River Formation where *Lycianthoides calycina* was collected (Garfield and Rio Blanco Counties, CO, USA). Yellow square on right, location of the Esmeraldas Formation where *Eophysaloides inflata* was collected (Eastern Cordillera, Santander, Colombia). Image taken from Paleoglobe for the Lutetian, *c.* 44.5 million years ago, by C. R. Scotese, PALEOMAP Project (Scotese & Dreher, 2012). Photographs by R. Deanna and C. Martínez. Bars, 2 mm.

Solanaceae from living material, herbarium specimens, and taxonomic literature. The extant taxa included at least one species per genus for the 99 genera of Solanaceae and several for large genera (e.g. *Solanum* L.) to capture fruit and fruiting calyx diversity across the family (Supporting Information Table S1). The fruit macrofossils included the holotype and paratype of *Physalis infinemundi* Wilf, the holotype of *P. hunickenii* Deanna, Wilf & Gandolfo, the holotype of *Eophysaloides inflata*, and the holotype and paratypes of *Lycianthoides calycina*. We applied a non-metric multidimensional scaling (NMDS) analysis to assess the similarity of the fossils to the extant taxa. This ordination approach, although most common in community ecology (Minchin, 1987), has become increasingly used for analyzing fossil diversity (Palazzesi & Barreda, 2012; Bush & Brame, 2016) and taxonomic affinities (Smith *et al.*, 2017; Herrera-Flórez *et al.*, 2020; Romero *et al.*, 2020) because it accommodates a mixture of discrete and continuous variables as well as missing data. All data were recorded in the PROTEUS database (Sauquet *et al.*, 2017), linking each record to an explicit source or voucher (Table S1). Using the final matrix of 298 species, we carried out NMDS with the metaMDS function and Gower's Distance as the dissimilarity metric in the VEGAN package in R (Oksanen *et al.*, 2013). All

characters were treated as symmetric except fruit type, which was considered ordered. We also ran a stress analysis to test how many dimensions are needed to plot the data. Results were plotted using the function ggplot from the R GGPLOT2 package (Wickham *et al.*, 2016).

Phylogenetic analysis

To complement the NDMS clustering analysis, we conducted a phylogenetic analysis of the combined morphological and molecular data. This combined dataset included all the extant Solanaceae species with available DNA sequences (265 of the 291 scored for NMDS) and the fossils already included in the morphospace analysis. Morphological data were the same as for NMDS, but the continuous traits were converted from mm to cm given the limitations of the software. We built the DNA dataset from Genbank using PyPHLAWD (Smith & Walker, 2018) as in the previous family-level analysis of Särkinen *et al.* (2013), but expanded the dataset to include one additional nuclear gene (LFY) and one additional plastid region (*ndhF-rpl32*; see list of partitions in Table S2; Dataset S1). Sequence alignments were performed using the MUSCLE algorithm (Edgar, 2004) in MEGA

v.11 (Tamura *et al.*, 2021) and manually adjusted to exclude ambiguous regions (see alignment in Dataset S1). We ran an initial maximum likelihood search in RAxML v.8 (Stamatakis, 2014) on this molecular dataset to identify any long branches or spurious relationships that could indicate errors in dataset construction (Fig. S1). We used the GTR + GAMMA substitution model for this partitioned maximum likelihood search and, to assess nodal support, we applied the rapid bootstrap (BS) algorithm with 1000 replicates (Fig. S1). After verifying recovery of the expected family-level relationships, we analyzed the combined dataset (Dataset S2) of DNA sequences and discrete and continuous morphological characters with parsimony, as it is the only method currently amenable to analysis of this combination of character types. We performed a traditional search using TNT v.1.5 (Goloboff *et al.*, 2008), with 1000 random sequence addition replicates, TBR branch swapping, and 10 trees saved per replicate. We rooted the tree with *Ipomoea batatas*, member of the Convolvulaceae family, and calculated decay indices (Bremer, 1994), relative Bremer support (Goloboff & Farris, 2001) and bootstrap values with 1000 replicates as measures of support. Finally, to identify synapomorphies uniting fossils and their closest living relatives, we also mapped each morphological character onto the most parsimonious trees in TNT (Goloboff *et al.*, 2008).

Results

Systematics

Order – Solanales Bercht. & J. Presl, 1820
Family – Solanaceae Juss., 1789, *nom. cons.*
Subfamily – Solanoideae Burnett, 1835
Tribe – Physalideae Miers, 1849
Genus – *Eophysaloides* Martínez-A. & Deanna, gen. nov.

Type species *Eophysaloides inflata* Martínez-A. & Deanna, gen. et sp. nov.

Etymology Eo − early; physaloides, a genus now synonymized with *Withania* Pauquy that includes species with inflated calyces similar to the fossil.

Diagnosis Small and inflated fruiting calyx surrounding a fleshy fruit (i.e. berry) that differs from extant and fossil *Physalis* in the smaller size, non-invaginated calyx base, and non-angled sides of the calyx. Also differs from *Lycianthoides* (see below) in its five-lobed inflated calyx with conspicuous primary veins and secondary veins that fork before reaching the lobe sinus.

Species *Eophysaloides inflata* Martínez-A. & Deanna, sp. nov. (Fig. 2a–c).

Etymology The proposed specific epithet refers to the notably inflated fruiting calyx in the fossil.

Holotype here designated STRI-SGC 36163. (Fig. 2a–c).

Type locality STRI-430134, Nuevo Mundo Syncline, Middle Magdalena Valley Basin, in the area of the Topocoro Dam near the road that leads from Bucaramanga to Barrancabermeja, Colombia.

Age and distribution Middle to late Eocene Esmeraldas Formation, Colombia. Dated at *c.* 47.3–33.9 Ma using biostratigraphic and chemostratigraphic correlations (Martínez *et al.*, 2021).

Repository Museo Paleontológico José Royo y Gómez, Colombian Geological Survey (SGC), Bogotá, Colombia.

Diagnosis As for the genus, due to monotypy (Art. 38.5, Turland *et al.*, 2018).

Description The species (Fig. 2a–c) is described based on one specimen of a compressed berry visible beneath a preserved surrounding calyx. The pedicel is 5.2 mm long and 0.6 mm wide, with an L : W ratio of 8.66. The calyx is strongly inflated, not basally invaginated, completely surrounding the berry, five-lobed, partly open at apex, 10.8 mm long, and 8.5 mm wide (L : W ratio 1.3 : 1). Calyx lobes are equally sized, with angular sinuses and incised one-sixth the total calyx length, tips acute, triangular. Venation consists of one robust primary meridional vein per lobe, terminating at the lobe apex and alternating with secondary veins. Secondary veins emerge from the calyx base, are visually distinct from the primaries and other vein orders, and fork close to the lobe sinuses. Intersecondary veins arise near the base, visually distinct from secondaries and tertiaries, dichotomizing into the random, irregular reticulum of tertiary through at least fourth-order veins that fill most of the vein field. The berry is ovoid, flattened, and coalified (i.e. converted into coal) from fossilization, 6.2 mm long and 3.2 mm wide, occupying half of the width of the inflated calyx; seeds not preserved.

Remarks We confirmed that the new genus and species *Eophysaloides inflata* belongs to Solanaceae by first comparing it with the 36 genera from 18 plant families that have inflated calyces; this list was compiled by experts among the authors and other angiosperm experts. Table S3 compares *E. inflata* with each of these extant taxa and describes the differences that exclude the fossil from these taxa. By comparison, *E. inflata* fits well within the Solanaceae due to having a berry encased in the inflated five-lobed membranous calyx, with an elongated pedicel and strong venation. Within Solanaceae, the fruit morphology resembles genera belonging to the Physalideae tribe, in particular *Calliphysalis* Whitson and *Brachistus* Miers (Fig. 2d–f). These genera share the presence of a berry loosely enveloped by a five-lobed inflated calyx that stands out by its non-invaginated base and distinctive venation pattern. The fossil here described cannot be assigned to any single living genus because *Brachistus* and *Calliphysalis* have morphological differences with the fossil and *Eophysaloides* is not nested in either of them (see phylogenetic analysis below). Therefore, we describe it as distinct at the generic level.

Fig. 2 *Eophysaloides inflata* gen. et sp. nov. and morphologically similar extant taxa. (a–c) Holotype of *E. inflata*, STRI-SGC 36163. (b) Photo-stack of *E. inflata*, STRI-SGC 36163. The thicker darkened portion, indicated with a black arrow in the center of (a, b), corresponds to a portion of the coalified fossil berry. Differences between (a, b) in contrast and color temperature are to denote venation pattern. (c) *E. inflata*, STRI-SGC 36163 (counterpart). Examples of similar extant taxa include (d) *Calliphysalis carpenterii* (Riddell) Whitson, USA, voucher West n.n. (BM000994243), (e) *Brachistus nelsonii* (Fernald) D'Arcy, J.L.Gentry & Averett, Belize, voucher Peña *et al*. 945 (BM000776481), (f) *Brachistus stramonifolius* Miers, Mexico, voucher Rzedowski 19 530 (CORD00014619). Like *E. inflata*, these taxa present inflated, five-lobed fruiting calyces with prominent secondary veins but lacking invagination at the base. (a–c) Photographs by C. Martínez, (d–f) by R. Deanna. Bars, 2 mm.
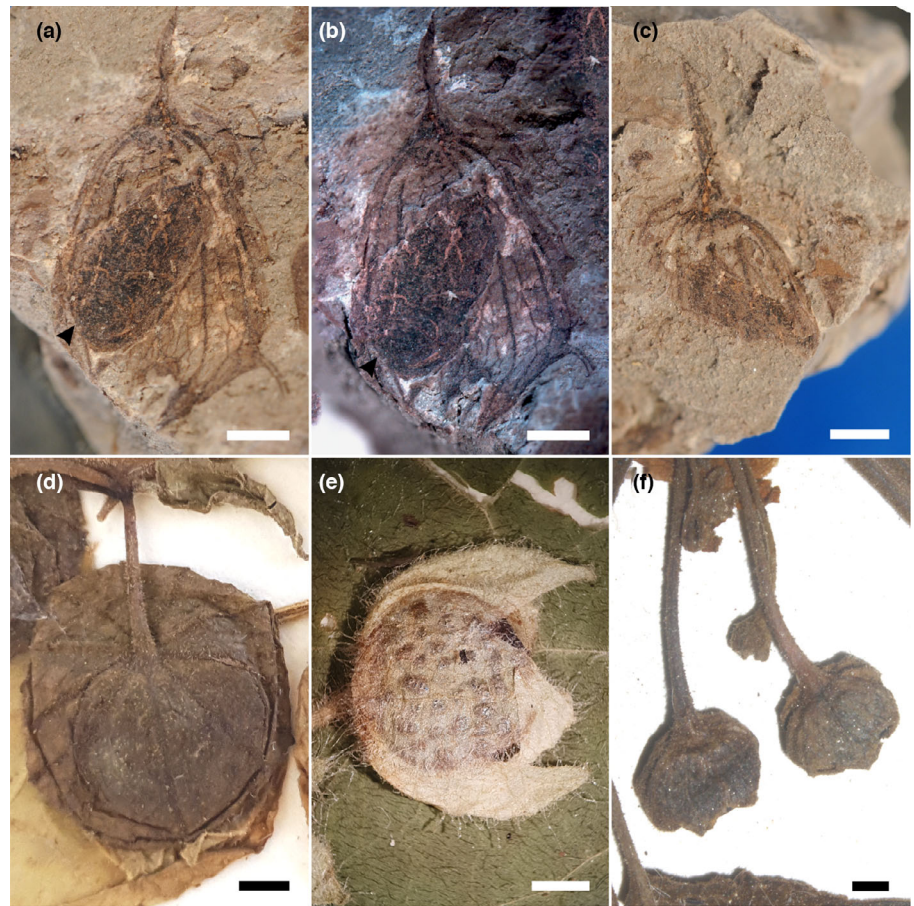
Order – Solanales Bercht. & J. Presl, 1820
Family – Solanaceae Juss., 1789, *nom. cons.*
Subfamily – Solanoideae Burnett, 1835
Tribe – Capsiceae Dumort., 1827
Genus – *Lycianthoides* Deanna & Manchester, gen. nov.

Type species *Lycianthoides calycina* Deanna & Manchester, gen. et sp. nov.

Etymology *Lycianthoides* refers to the morphological resemblance of the fossil to *Lycianthes* (Dunal) Hassl. and relatives in the tribe Capsiceae (e.g. *Capsicum* L.).

Generic diagnosis As for the species, due to monotypy (Art. 38.5; Turland *et al.*, 2018).

Species *Lycianthoides calycina* Deanna & Manchester, sp. nov.

Etymology The proposed specific epithet highlights the distinctive calyx preserved in this fossil.

Holotype here designated UCM 41276a,b (Fig. 3a–c).

Paratypes UCM 41285 (Fig. 3d–f); DMNH EPI.57889a,b (Fig. 3g,h).

Type localities Holotype: Claudia's Place (UCM locality 20099063), Green River Formation, Garfield County, CO, USA. Paratype UCM 41285: Anvil Points locality (UCM loc. 2005026), Green River Formation, Garfield County, CO, USA. Paratype DMNH EPI.57889a,b: Ron Meyer's Scorpion (DMNH loc. 304), Green River Formation, Rio Blanco County, CO, USA.

Age and distribution All specimens from the early Eocene Green River Formation, western United States, from strata constrained to *c.* 49.5–51.5 Ma from ^{40}Ar/^{39}Ar analyses (Smith *et al.*, 2008; Smith & Carroll, 2015).

Repositories. Holotype UCM 41276a,b and paratype UCM 41285 at the University of Colorado Museum (UCM), Paleontological Section, Boulder, CO, USA of America. Paratype DMNH EPI.57889a,b at Denver Museum of Nature & Science, Denver, CO, USA.

Specific diagnosis *Lycianthoides calycina* differs from the fossils *Physalis infinemundi*, *P. hunickenii*, and *Eophysaloides inflata* in its non-inflated calyx with prominent, finger-like appendages and its venation pattern, wherein secondary veins extend from the base of the calyx to the primary vein tips, giving rise to a reticulum of thinner tertiary veins. *Lycianthoides* also differs from extant
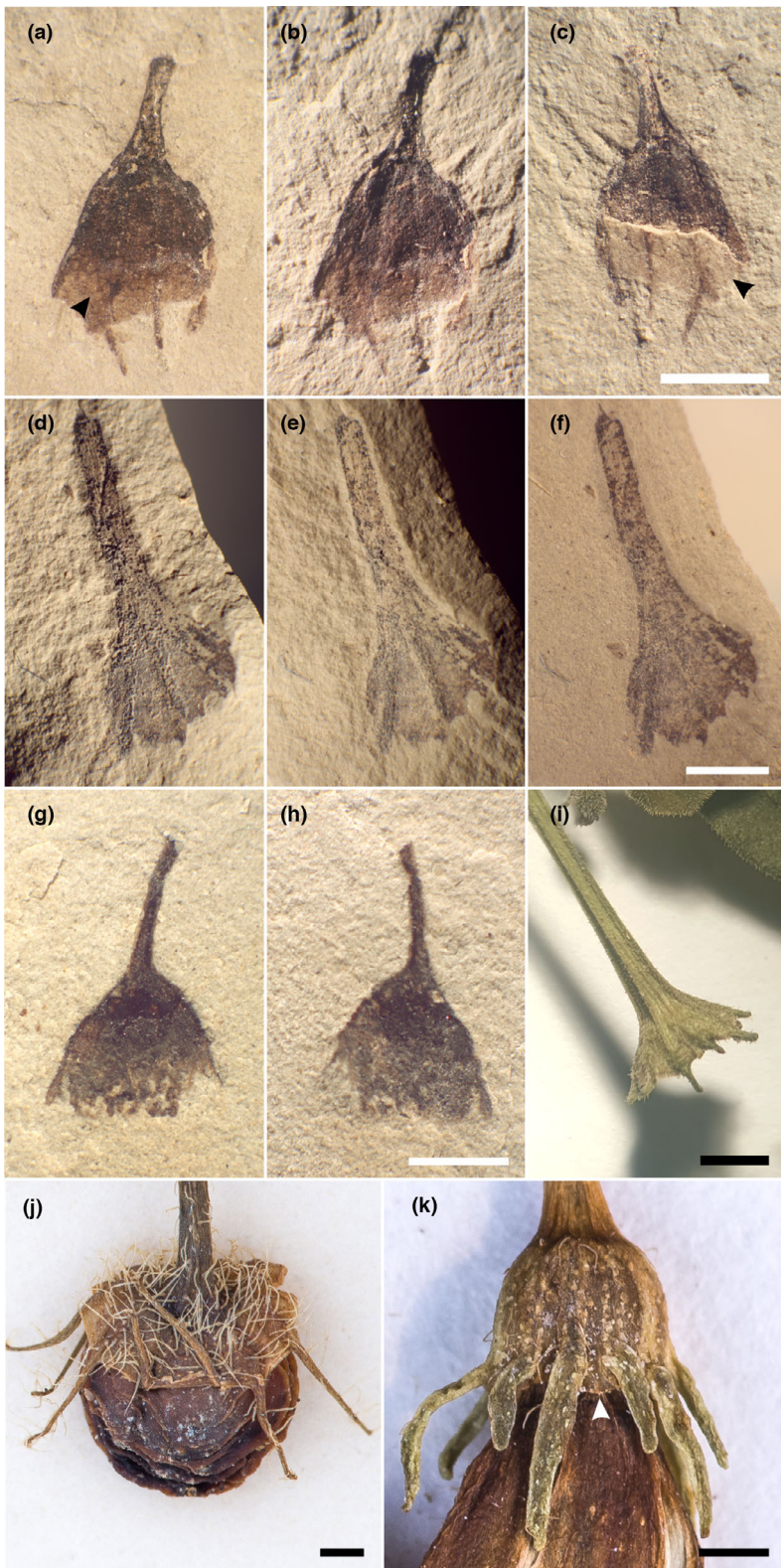
Fig. 3 *Lycianthoides calycina* gen. et sp. nov. and morphologically similar extant taxa, under microscopy with varying lighting. (a–c) Holotype of *L. calycina*, UCM41276a (a, b), UCM41276b (c). (d–f) Paratype of *L. calycina*, UCM41285. (g) Paratype of *L. calycina*, DMNH EPI.57889a. (h) DMNH EPI.57889b. (i) *Capsicum baccatum*, Bolivia, voucher Cocucci & Sérsic 5488 (CORD00082326). (j) *Lycianthes amatitlanensis* Bitter, Guatemala, voucher von Türckheim 7753 (US01269193). (k) *L. venturana* E.Dean, Mexico, voucher Tenorio 11 426 (MEXU611648). Like *L. calycina*, these taxa present strongly veined but not inflated calyces with distinctive finger-like teeth and a pedicel that widens at the point of insertion. Arrow in (a) indicates the secondary veins that came from primary veins tips to the base of the calyx, and arrows in (c, k) indicate the calyx rim that extends beyond the insertion of the teeth. (a–f, i) Photographs by R. Deanna, (g, h) by S. Manchester, (j, k) by D. McNair. Bars: (a–j) 2 mm; (k) 1 mm.

Lycianthes and *Capsicum* in its smaller size and usually thinner finger-like appendages (Fig. 3i–k).

Description The holotype (Fig. 3a–c) displays a typical *Lycianthes/Capsicum* morphology (Fig. 3i). It comprises a compressed calyx, length 2.7 mm, width 2.9 mm, with an estimated L : W ratio of 0.9. The rounded shape of the calyx suggests the presence of a developing fruit. The pedicel is partially preserved, 1.9 mm length, 0.3 mm width, with an estimated L : W ratio of 5.6 and expands in width toward the calyx to 0.8 mm at

the point of junction with the calyx. The calyx is not basally invaginated, not inflated, widely open at the apex, and without lobes but with 8–10 submarginal teeth (appendages), each 1.1–1.7 mm long and 0.1 mm wide. Calyx appendages are unequally sized, narrowly awl-shaped, inserted *c.* 0.7 mm from the calyx rim to almost one-fourth the total calyx length. Venation includes one robust primary meridional vein per appendage, terminating at the apex. Secondary veins are visually distinct from the primaries and other vein orders, emerge from the appendage apices and are directed toward the base of the calyx. Tertiary veins are distinctive, come from the secondary and form a distinct reticulum between the calyx appendages.

Paratype UCM 41285 (Fig. 3d–f) also represents a compressed calyx, very similar to the holotype, but with poorer preservation. The calyx is 2.9 mm long and 2.5 mm wide, with an estimated L : W ratio of 1.1. The pedicel is partially preserved, 4.9 mm long and 0.8 mm wide, increasing in width toward the calyx to 1.1 mm at the junction. This specimen differs from the holotype in the lack of preservation of secondary veins and the partial preservation of the calyx appendages. Five conspicuous primary veins run from the pedicel to the calyx margins. Secondary and tertiary veins are not distinguishable.

Paratype DMNH EPI.57889a,b (Fig. 3g,h) is similar to the other two specimens, consisting of a compressed calyx with inconspicuous teeth. It differs in its much smaller size, length 1.1 mm, width 1.2 mm, with an estimated L : W ratio of 0.9. The calyx base is not invaginated and the preservation of calyx appendages is very poor. Neither primary nor secondary veins are identifiable.

Remarks The new genus and species *Lycianthoides calycina* is assignable to Solanaceae after a careful comparison with other angiosperms and consultation with experts in other plant families. We identified only three other angiosperm taxa with similar calyx appendages starting above the calyx edge, and in each case, other characters exclude the fossil from those families (Table S3). The Solanaceae is the only family that has species with the combination of a truncate calyx (having no lobes) and tooth-like appendages, and the only extant species with these features belong to *Capsicum* and *Lycianthes* (Figs 3i–k). The three fossils all share submarginal calyx appendages forming awl-shaped teeth, the rounded shape of the calyx base, and the broadening of the pedicel on the insertion with the calyx. The new fossil taxon cannot be assigned to a particular living genus because these traits are found in both *Capsicum* and *Lycianthes* and the fossil is also morphologically different to both, thus we described it in a new genus. Among the three specimens, there is variation in preservation, especially of appendages and venation; the holotype is the specimen with the most conspicuous net-like venation. However, all specimens possess the same calyx shape and pedicel features, supporting their description as a single species.

Morphospace of fossil and extant nightshade fruits

In our NDMS analysis, all 15 morphological characters were significantly informative ($P = 0.001$–0.005), except for the presence of calyx appendages (Table S4). However, we retained this character because of the importance of calyx appendages in diagnosing members of the *Capsicum*/*Lycianthes* clade (Fig. 3). A stress analysis (measuring goodness-of-fit) showed that two dimensions produce a stress value < 0.2, which indicates that the plot is interpretable (Fig. 4). As expected from earlier results (Wilf *et al.*, 2017; Deanna *et al.*, 2020), the *Physalis* fossils (Fig. 4a–c) from Patagonia grouped with extant *Physalis*, *Alkekengi* Mill., and *Physaliastrum* Makino (Fig. 4d,e), which share an inflated calyx, invagination where the pedicel joins the fruiting calyx, and similar venation patterns. The distances among samples in ordination space support similarities between *Eophysaloides inflata* (Fig. 4f) and *Brachistus* (Fig. 4g) as well as the monotypic *Calliphysalis*, whose common features include the non-invaginated base of an inflated calyx and a distinctive venation pattern. *Lycianthoides calycina* from Colorado (Fig. 4h) clusters with extant *Lycianthes* and *Capsicum* (Fig. 4i,j), genera that share its submarginal awl-shaped appendages on a non-inflated calyx with no lobes, in addition to conspicuous and netlike venation.

Phylogenetic placement of fossils

The total evidence matrix included 3806 parsimony-informative characters (10 morphological discrete, five morphological continuous, 3791 molecular). Parsimony analysis in TNT recovered two most parsimonious trees of 19 666 steps; one of these two is shown as a phylogram in Fig. S2. As these two trees differed only the position of one *Solanum* species, the strict consensus of these two trees was almost fully resolved (Fig. 5a) and suggested similar taxonomic affinities as the NMDS analyses (Fig. 5b,c). The decay indices, relative Bremer, and Bootstrap supports were low for most branches (Figs S3–S5), although several character changes were identified that unite each fossil with different berry clade lineages (Fig. S6). The *Physalis* fossils and *Eophysaloides inflata* were inferred to belong in the Physalidinae subtribe of Physalideae due to the increase in the length of the fruiting calyx (0.5–1.1 to 1.3–1.38 cm, character 0 in Fig. S6) and the increase in the length of the calyx lobes (0.18–0.28 to 0.36–0.44 cm, character 2 in Fig. S6). Within this clade, all *Physalis* fossils clustered with *Alkekengi officinarum* because they share an invaginated and angled calyx (characters 5 and 6, Fig. S6) and show large increases in calyx length (1.55 to 2.16 cm), fruiting calyx length–width ratio (0.11–0.12), and calyx lobe length (0.36–0.44 to 0.79 cm) (characters 0, 1, and 2, Fig. S6). *Eophysaloides* grouped with *Brachistus nelsonii* by the presence of calyx secondary veins forking before lobe sinus (character 11, Fig. S6), which together with the lack of invagination at the base of the calyx, presents a unique character combination within the family. Although *Lycianthoides calycina* does not appear to be monophyletic in the parsimony analysis, possibly due to the missing data for the two paratypes, all three specimens are placed within the Capsiceae tribe (Fig. 5) by the absence of calyx lobes, the gain of calyx teeth, and the lack of major calyx veins that terminate in lobe tips (characters 2, 8 and 9, Fig. S6). The loss of calyx lobes is homoplasious (e.g. *Tubocapsicum* Makino and some *Solanum* have truncate
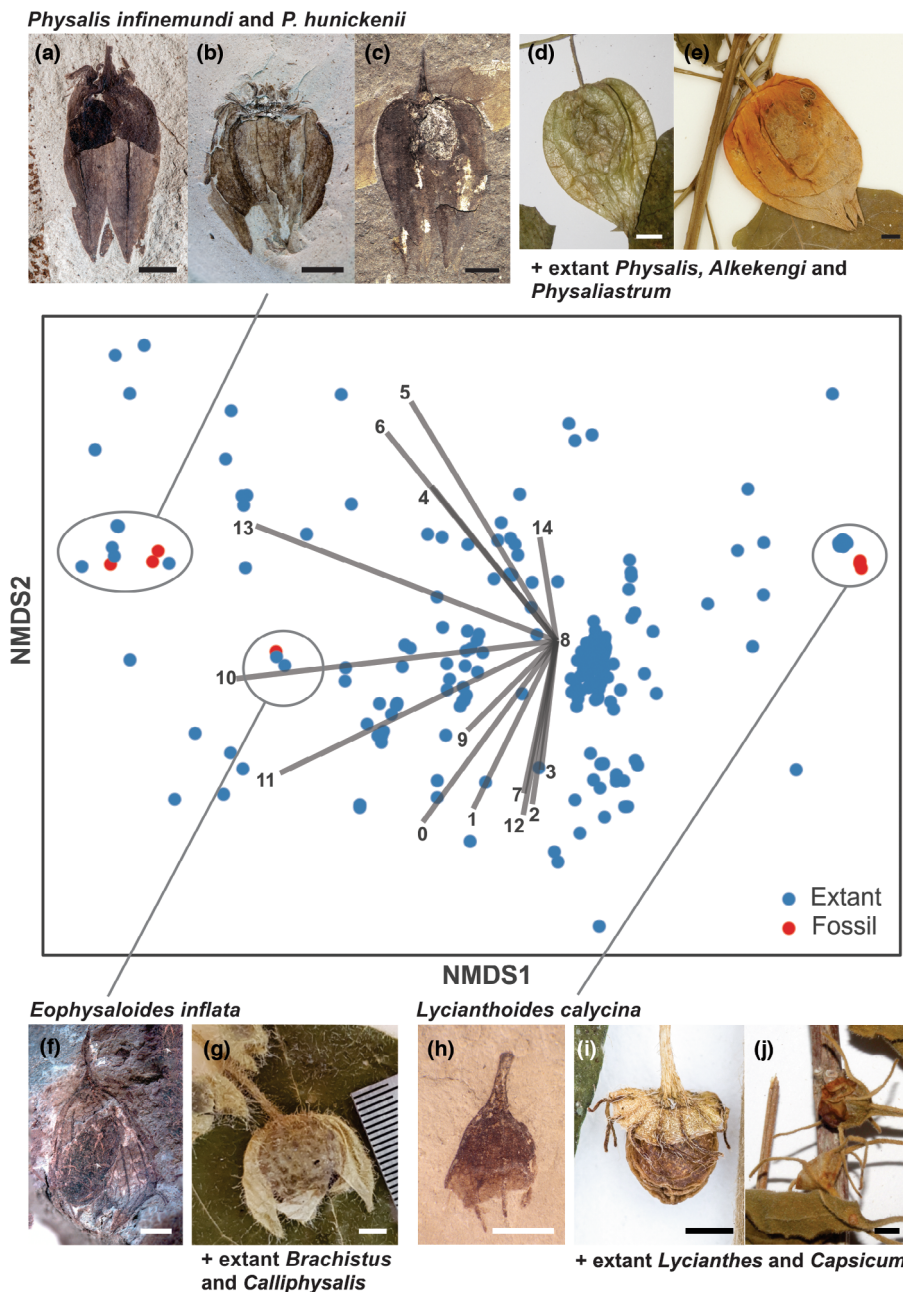
Physalis infinemundi and **P. hunickenii**

+ extant **Physalis, Alkekengi** and **Physaliastrum**

Eophysaloides inflata

+ extant **Brachistus** and **Calliphysalis**

Lycianthoides calycina

+ extant **Lycianthes** and **Capsicum**

Fig. 4 Non-metric multidimensional scaling (NMDS) analysis of 15 morphological characters from seven fossil reports and 291 extant species of Solanaceae, with three resulting groupings of fossil and extant taxa indicated. (a) Holotype of *Physalis infinemundi*, MPEF-Pb 6434a. (b) Paratype of *P. infinemundi*, MPEF-Pb 6435a. (c) *P. hunickenii* holotype, MPEF-Pb 6443a. (d) *Physalis angulata*, Peru, voucher Barboza *et al*. 4982 (CORD). (e) *Alkekengi officinarum*, USA, voucher Hill 25 957 (NY01455682). (f) *Eophysaloides inflata* holotype, STRI-SGC 36163. (g) *Brachistus nelsonii*, Belize, voucher Peña *et al*. 945 (BM000776481). (h) *Lycianthoides calycina* holotype, UCM41276a. (i) *Lycianthes inaequilatera*, Peru, voucher Iltis 303 (US2848803). (j) *Capsicum longidentatum*, Brazil, voucher Agra *et al*. 7086 (CORD). (a, b) Photographs by P. Wilf, (c, d, g, h, j) by R. Deanna, (e) image courtesy of the C. V. Starr Virtual Herbarium (http://sweetgum.nybg.org/science/vh/), (f) by C. Martínez, (i) by D. McNair. Bars: (a–e) 5 mm; (f–j) 2 mm. Characters: 0, fruiting calyx length; 1, fruiting calyx length : width ratio; 2, length of calyx lobes; 3, calyx lobe length : width ratio; 4, ratio between calyx total length to lobes length; 5, presence/absence of invaginated calyx base; 6, presence/absence of angled calyx; 7, sinus rounded or angled; 8, presence/absence of calyx teeth; 9, presence/absence of calyx widest veins distinctive of other vein orders that terminate in lobe tips; 10, presence/absence of calyx secondary veins distinct from other vein orders that emerge from base; 11, presence/absence of calyx secondary veins forking before lobe sinus; 12, fruit type (berry, capsule, diclesium, drupe, mericarp, pyxidium or non-capsular dehiscent fruit); 13, presence/absence of inflated calyx; 14, calyx venation pattern (parallelodromous or other type).

calyces), but the submarginal teeth are a synapomorphy of Capsiceae (character 8, Fig. S6).

Discussion

Fossils from three berry clade lineages lay out a new timeline for nightshades

With the rich set of characters preserved in the suite of Eocene fruit macrofossils, we have uncovered three distinct fossil lineages belonging to different groups within the nightshade family. The two previously described lantern fruit fossils from Argentina fall clearly within the cluster (Fig. 4) containing extant *Physalis* and two closely related genera (*Physaliastrum* and *Alkekengi*; see also Wilf *et al*., 2017, Deanna *et al*., 2020, who both noted additional similarities with *Physalis*), while the new Colombian fossil *Eophysaloides inflata* groups with other genera of tomatillo subtribe Physalidinae (*Brachistus* and *Calliphysalis*). In addition to these physaloid fossils, the newly discovered fossil from the Eocene of Colorado, *Lycianthoides calycina*, belongs to the chilipepper tribe Capsiceae, which includes the genera *Lycianthes* and *Capsicum*. Taxa in this clade present distinctive calyces, often with a truncated (entire) margin and with or without elongated and showy marginal or submarginal appendages. *Lycianthoides calycina* closely resembles extant species from both the Americas and Asia in the shape and size of these elongated appendages (Fig. 3). Although the available fruit fossils are still outnumbered by the putative Solanaceae seed fossils, the paucity of characters in seeds
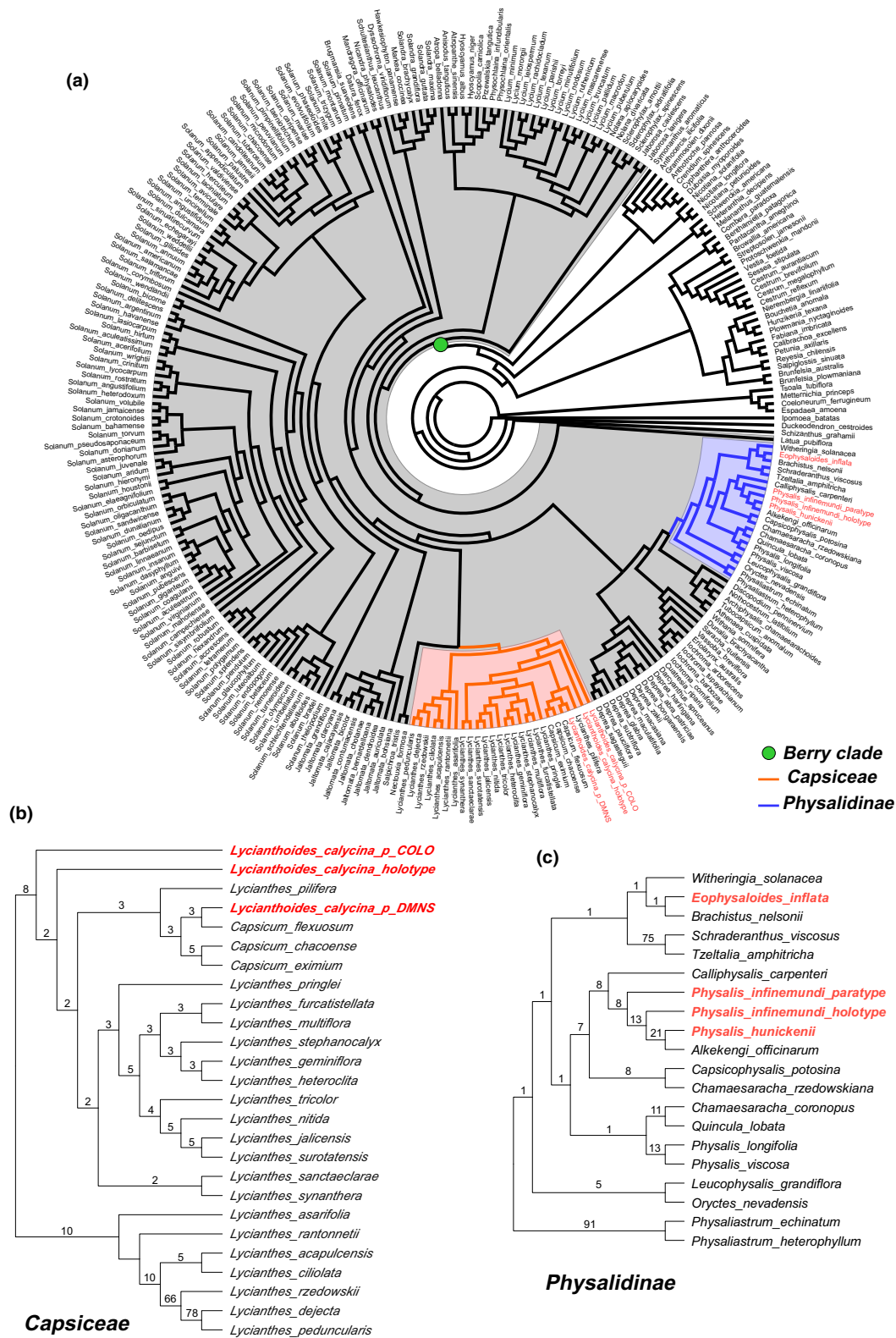
Fig. 5 Maximum parsimony phylogenetic analysis. (a) Strict consensus of the two most parsimonious trees. (b) Strict consensus of the Capsiceae subtribe including the relative Bremer support above the branches. (c) Strict consensus of the Physalidinae subtribe including the relative Bremer support above the branches. All bolded names in red correspond to fossils. Grey shading and green dot in (a) correspond to the berry clade.

has made it difficult to compare them with living taxa and to assign them to particular clades of the family (Särkinen *et al.*, 2013, 2018; Millan & Crepet, 2014). Thus, our study

represents a major advance in not only describing two new fossil fruits, but also in leveraging their well-preserved features to infer their placement within major lineages of the solanaceous berry

clade. Given that the previously discovered Eocene fruit fossils were both from a single site in Argentina (Wilf *et al.*, 2017; Deanna *et al.*, 2020), the new fruit fossils from Colombia and Colorado greatly expand the known geographic distribution of the family during this time period.

Given their affinities, age, and distant locations, these fruit fossils suggest a much older timeline for the diversification and global dispersal of the nightshade family and its component berry clade. Previous divergence time analyses estimated crown ages of *c.* 10 and 15 Ma for Physalidinae and Capsiceae, respectively, and 26–68 Ma for the entire Solanaceae family, depending on the fossils and methods used (Särkinen *et al.*, 2013; Zamora-Tavares *et al.*, 2016; De-Silva *et al.*, 2017; Ramírez-Barahona *et al.*, 2020). The fossil fruits described here trace to the Eocene, including the early Eocene, indicating that the Capsiceae as well as the Physalideae (Wilf *et al.*, 2017; Deanna *et al.*, 2020) are three to four times older than previously estimated. Their distribution (from modern-day South Argentina to Colombia and Colorado), along with prior seed records from the Eocene of Europe and Oregon (Chandler, 1962; Manchester, 1994), shows that the family was well diversified and geographically widespread by the Eocene (56–33.9 Ma). Although we lack older fruit fossils that could validate the inferred ancestral state for the family (a capsule, Knapp, 2002) and anchor the basal nodes, we suspect that the crown age for the Solanaceae falls in the Paleocene (66–56 Ma) or the Late Cretaceous (100–66 Ma), similar to other Gondwanan plant families with high present-day diversity in the Andes (Antonelli *et al.*, 2009; Barreda *et al.*, 2015; Mandel *et al.*, 2019; Carvalho *et al.*, 2021; Serna-Sánchez *et al.*, 2021).

The fossil discoveries described here lay the core foundation for building a new time tree for the family with total-evidence approaches. While there have been recent advances in model-based divergence time estimation, in particular the development of the fossilized birth–death model (Heath *et al.*, 2014; Zhang *et al.*, 2016), current implementations do not accommodate quantitative characters. Morphometrics are often key in fossil placement, especially for plant material with relatively few scorable qualitative characters, like pollen and seeds (e.g. Gong *et al.*, 2010; Romero *et al.*, 2020). We found that quantitative characters, such as the size of the calyx and its lobes, were the most informative for identifying relationships between the fruit macrofossils and living material, although assessing the statistical robustness of these relationships is limited by the parsimony framework we applied. Theoretical work is advancing toward incorporating morphometrics into model-based divergence time estimation (Parins-Fukuchi, 2018a,b), suggesting that a total-evidencing dating, including discrete and continuous characters for extinct and extant taxa along with molecular sequence data, will soon be available. The application of model-based total evidence approaches to Solanaceae will provide a clearer picture of the uncertainty around relationships between fossil and extant taxa (e.g. Lee & Yates, 2018; May *et al.*, 2021) and may suggest alternative placement for the fossils described here. Nevertheless, with previous analyses using the seed fossils and *Physalis* fruit fossil only for node-dating, we expect that full integration of the fossils into divergence time estimation will significantly push nodes deeper in time across the phylogeny and may have implications for the broader angiosperm timetree.

Fleshy-fruited nightshades suggest new routes for spread during the Late Cretaceous

The wide distribution of Eocene nightshades and the possibility of the family's diversification in the Late Cretaceous and early Paleogene open several new possibilities for the group's potential routes and mechanisms of spread around the globe. Using previous molecular dating estimates, biogeographic studies have estimated a minimum of 20 transoceanic dispersals, all from South America and within the Neogene (Olmstead, 2013; Dupin *et al.*, 2017). Because the fruit fossils now show that the Solanaceae are much older and diversified in part on Gondwana, transoceanic dispersals may not be necessary to explain modern distributions. Today's fleshy tomatillos and peppers are animal dispersed (Tewksbury & Nabhan, 2001; Knapp, 2002), and this feature may have promoted their spread, aligning with the well-known radiations of frugivores and large-fruited angiosperms during the early Paleogene (66–56 Ma; Benton *et al.*, 2022). Biotic exchange between the Americas could have occurred via short distance over-water dispersals or along the ephemeral chains of Late Cretaceous Caribbean volcanic islands (100–66 Ma; Iturralde-Vinent, 2006; Wilf *et al.*, 2013; Roncal *et al.*, 2020). The Gondwana connections may have also facilitated expansion into Australia and Asia, a scenario consistent with the deep splits between Australasian and American lineages in several nightshade clades (e.g. Nicotianoideae, Garcia & Olmstead, 2003; Capsiceae, Spalink *et al.*, 2018).

Our discovery of a North American fruit fossil also points to the potential for early dispersal of nightshades across the Northern Hemisphere through Holarctic land connections when those areas were much warmer than today (Manchester, 1999; Brikiatis, 2014). The Solanaceae fossil seed record supports this northern spread during the middle to late Paleogene (47.8–23 Ma), with specimens assigned to the berry clade spanning present-day Oregon (43.8 Ma, Nut Beds Flora, Manchester, 1994), northern Europe (*c.* 45 Ma, Poole Formation, Chandler, 1962), and Russia (23–16 Ma, Western Siberia, Dorofeev, 1963). Despite this broad distribution in the Northern Hemisphere 40–50 million years ago (Ma), the family remains comparatively depauperate in temperate regions worldwide today, with only 13% of the extant species diversity outside the tropics (Dupin *et al.*, 2017). For example, there are no native species of *Capsicum* or *Lycianthes* in the region of the Rocky Mountains where *Lycianthoides* was found. It is possible that the solanaceous flora of western North America experienced the same fate as many other American thermophilic groups that expanded in that region during the Eocene but then retreated southward as the climate subsequently became cooler and drier (Leopold & MacGinitie, 1972; Wing, 1987; Meyer & Manchester, 1997; Daubenmire, 2012). Meanwhile, the phases of Andean uplift in South America may have contributed to radiations within major berry lineages, such as the physaloids, solanums, and peppers, which our results indicate were all present well before the modern orogeny began during the Neogene (23–2.6 Ma).

Competing interests

None declared.

Author contributions

RD and SDS conceptualized the original idea, wrote the original draft, reviewed, and edited; SDS supervised the planning and execution of the research; CM, RD and AC found the fossils and wrote the descriptions and geological setting; RD, CM and SM took photographs of the new fossils, RD, SK, FEC, ED, GB, GEB, SDS and AB scored morphological traits; HS designed and managed the platform including the morphological traits; RD analyzed the data and prepared the figures; CM, SM, PW, SK, FEC, GEB, GB, HS, ED and AO reviewed and edited the drafts.

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Data availability

All data used in this study are included in the article and/or Supporting Information.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Alignment of the DNA sequence data used in the phylogenetic analyses.

Dataset S2 TNT file containing continuous, discrete and DNA characters.

Fig. S1 Maximum likelihood tree obtained of the combined dataset with all the molecular markers.

Fig. S2 One of the two most parsimonious phylograms obtained of the combined dataset with morphology and all the molecular markers.

Fig. S3 Strict consensus of the two most parsimonious trees including decay indices (absolute Bremer support) above branches.

Fig. S4 Strict consensus of the two most parsimonious trees including relative Bremer support above branches.

Fig. S5 Strict consensus of the two most parsimonious trees including bootstrap support above branches.

Fig. S6 Strict consensus of the two most parsimonious trees including morphological synapomorphies above branches.

Table S1 Dataset of 10 discrete and 5 continuous characters for seven fossils and 291 extant species of Solanaceae including voucher or bibliography information for each character scored.

Table S2 Partitions of DNA sequence alignment used in phylogenetic analysis.

Table S3 Similarities and differences of other genera of angiosperms than Solanaceae to both fossil species, *Eophysaloides inflata* in the first tab and *Lycianthoides calycina* in the second tab.

Table S4 Discrete and continuous characters analyzed including scores that show contribution of each variable to each axis of the NMDS and significance.

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