Elephants develop wrinkles through both form & function

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Abstract

Elephant trunks have folds and wrinkles, but we don't know how wrinkles differ between elephant species and how trunks and their wrinkles develop. Adult Asian elephants have significantly more dorsal major trunk wrinkles (~126 ± 25 SD) than African elephants (~83 ± 13 SD). There are more dorsal than ventral major trunk wrinkles and there is a closer wrinkle spacing distally than proximally. Wrinkle numbers differed slightly as a function of trunk-lateralization. MicroCT-imaging revealed a relatively constant thickness of the outer elephant trunk skin, whereas the inner skin parts are thicker between folds than in folds. The trunk shows the greatest fetal length growth of elephant body parts. Trunk wrinkles are added in an early exponential phase, where wrinkles double every 20 days, and a later phase, where wrinkles are added slowly and at a faster rate in Asian compared to African elephants. We suggest wrinkles improve the ability of trunk skin to bend.

Introduction

Elephant trunks are described as one of the three prominent examples of muscular hydrostats along with octopuses' arms and reptile or mammalian tongues[1–3]. A key aspect of the functionality of the elephant trunk is the immense motility and flexibility of this organ. Such motility is supported by a highly complex musculature[4–6], which is controled by a very elaborate motor nucleus[7]. The trunk is not a homogeneous structure, but differs in functionality across the proximal-distal axis. Specifically, the distal parts of the trunk are very dexterous, whereas proximal trunk regions play a lesser role in manipulation. Trunk function becomes lateralized in the course of elephant development and adult elephants split into left- or right-trunkers according to their grasping preferences[8–10]. In a previous study we found that so-called left-trunkers, who preferentially grasp towards the left side, have shorter whiskers of the right trunk side[11]; the reverse is true for right-trunkers. Such whisker length differences presumably are the result of differential whisker abrasion and they allow to determine trunk lateralization from trunks post mortem[11]. Trunks also differ morphologically and functionally between elephant species. African savanna elephants (*Loxodonta africana*, from here on called African elephants) and African forest elephants (*Loxodonta cyclotis*, not subject of this publication) have two finger-like protrusions on their trunk-tip and tend to pinch objects with their two fingers. Asian elephants (*Elephas maximus*) in contrast, have only one dorsal trunk finger and tend to wrap their trunk around objects[12].

In addition to the form of the trunk, there are functional specializations of the appendage. Neither elephant species has sweat glands due to their aquatic origin[13]. Therefore, the trunk can be used to suction up fluids such as water or mud to throw on the back of the elephant to remain cool[14]. The tough and dense skin protecting the body of the elephant from exposure to the elements is cracked for thermoregulation[15]. This tough skin has flexibility by having collagen fiber arranged in all directions allowing for a combination of strength and flexibility[16].The integumentum (outer layer) of the trunk, however, is densely packed with wrinkles that eventually transition to folds as you approach the proximal portion[17]. The formation and function of these wrinkles are thought to be for extension on the dorsal side[17] and grip on the ventral side[18]. The creases of elephant skin have already been described around 50 years ago[19] and their skin has been studied to some extent, but the anatomical and gross morphology of the development of the wrinkles remains unexplored.

Various animals have wrinkled substrates on their bodies with some common examples being that of the elephant trunk. One of the unique features of skin wrinkling is that it is an example of a physical instability. Elephants have wrinkles from birth and for commonly made materials that are layered like skin, wrinkles normally form if an outside stress has caused the top layer of a material to wrinkle causing an instability[20]. The understanding of how these wrinkles develop and change over time can help provide insight into biological instabilities and how they form in the natural world[21]. In our analysis, we aimed at elucidating functional and developmental characteristics of elephant trunk wrinkles. Specifically, we ask: (1) What is the number and distribution of wrinkles over the trunk in adult elephants, calves/fetuses and across elephant species? (2) Are elephant's trunk wrinkles affected by trunk use and lateralization? (3) What is the skin structure underlying elephant trunk wrinkles? (4) How do elephant trunks and trunk wrinkles develop?

Materials and Methods

Elephant specimens

All post-mortem specimens used in this study came from zoo elephants and were collected by the IZW (Leibniz Institute for Zoo and Wildlife Research, Berlin) over the last three decades in agreement with CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) regulations. Specimen reports and CITES documentation for all animals included are held at the IZW. All of these elephants had died of natural causes or were euthanized by experienced zoo veterinarians for humanitarian reasons, because of insurmountable health complications. Most of the trunks used were either fixed in 4% formaldehyde solution or frozen at -20°C. Table 1 gives an overview of the post-mortem specimens of Asian elephants (*Elephas maximus*) and African elephants (*Loxodonta africana*), along with the age.

In addition to these photographs of elephants in zoos were analyzed, Table 2 gives an overview of the elephants that were subjects here. Photographs were either taken by one of the authors at the Berlin Zoo and the Zoo Schönbrunn, Vienna, or provided by zoo employees, collaborators or photographers.

Photography of trunks

Post-mortem specimen at the lab and elephants at the zoos were photographed using a Sony α 7R III camera or a Sony α 7S II with a Sony FE 16-35mm F2.8 GM E-Mount objective or a Sony FE 90 Mm/2.8 Macro G OSS objective. Cameras were used handheld or mounted on a Hama "Star 62" tripod or a Manfrotto "MT190CXPRO4" carbon tripod.

Elephant wrinkle measurements

For counting, we adjusted color, sharpness, and contrast of the photographs in Adobe Photoshop (Adobe Systems Incorporated) for maximum visibility of wrinkles. The trunks were divided into zones: base, lateral shaft, dorsal part of the shaft, and tip (Figure S1). Wrinkles were identified as either “major” or “minor” wrinkle, with the first being deeper, mostly regularly spaced, and transversing the whole dorsal or ventral part of the shaft (Figure 2A and B). Deep skin folds as described previously[17] were only observed in the African elephants and for our analysis counted as “major” wrinkles. “Minor” wrinkles were defined as obviously shallow, sometimes not crossing all of the dorsal part of the shaft, but always at least 50 %, and few of them had gaps and were only counted if it was clear where they continued. Trunk zones (Figure S1) and wrinkles (Figure 2A, B, F) were drawn on the photographs using Adobe Photoshop and then counted manually using the multi-point tool in ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA.

To determine the position of the wrinkles on a trunk, ImageJ was used to measure the distance between wrinkles. A straight line between the eyes of the elephant was drawn as the zero line. The wrinkle distance was measured from the previous wrinkle or the zero line if it was the first wrinkle. Only the wrinkles distal of the zero line were plotted. The position of the wrinkles was normalized to the sum of all the measurements from the zero line until the tip of the dorsal trunk finger. The position of wrinkles in percent of the trunk (Figure 2D) was used to plot heatmaps showing the distribution of wrinkles along an elephant trunk (Figure 2E).

For the lateralization we counted the lateral wrinkles directly on the trunk tips available in the lab. The criteria for wrinkle classification were the same to the dorsal wrinkle methodology. Additionally, the wrinkles were draw over on Adobe Photoshop and counted with ImageJ. We counted the wrinkles on both side and for the first 15cm on the shaft, after the end of the dorsal tip.

Fetal development

To study trunk wrinkle and trunk development, we studied five fetal elephant specimens (n = 3 African elephant fetuses from the Naturkundemuseum Berlin, n = 2 Asian elephant fetuses from our own collection). We also made a major effort to collect photographs or drawings of elephant fetuses (n = 50 African, n = 12 Asian) from published work. Specifically, we obtained trunk photographs / drawings from the following references[13,22–47]. We assigned presumed embryonic ages (in days) according to the formulas for embryonic length[36] or mass[36] in early fetuses. In older fetuses (> E200) we used the mass-age formula developed by Craig 1984[29].

MicroCT scanning

All samples for microCT scanning were taken from trunks that were fixed in 4% formaldehyde for several months. To characterize wrinkles from different trunk regions, an Asian baby elephant trunk was cut in half sagittal and stained in 1% iodine solution for 33 days to enhance tissue contrast. The half trunk was then stained for 84 days in a lower concentration of Iodine solution. For the African baby elephant trunk, the sample was first put 30 days in 1% iodine solution, 30 days in 2% iodine solution and finally 30 days in 3% iodine solution.

All iodine solutions were prepared by diluting 5% Lugol's iodine in distilled water. The scans for the Asian baby elephant trunk were performed using our institute's YXLON FF20 CT scanner (YXLON International GmbH, Hamburg, Germany). The African Baby trunk was scanned at the Museum für Naturkunde Berlin with a YXLON FF 85 CT (YXLON International GmbH, Hamburg, Germany).

MicroCT determining trunk wrinkle and amplitude

The amplitudes of the trunk wrinkles were taken as a trough-to-peak measurement as in a sinusoidal wave. The amplitude was calculated using side views of transversely dissected trunks allowing peak-to-peak calculation of various segments along the trunk's surface.

The wavelength of the trunk wrinkles was taken as the distance between any two wrinkles. The wrinkles were sketched as tangential lines. To calculate the wavelength and the wrinkle number, perpendicular lines were drawn from the left side to the right side of the trunk. The number of intersections was described as a wrinkle number for that segment, and the distance between each intersection is the wavelength between those wrinkles. We analyzed all the zones previously described for the average wavelength between wrinkles and the wrinkle number along the trunk.

To compare between the African and Asian elephant microCTs (Figure 4A & Figure 4E) we normalized the positional information using the total trunk length. Therefore, the values for the trunk wrinkle amplitude & wavelength are plotted on the same axis of African and Asian elephants by dividing the position along the trunk by total length

This means the trunk position is unitless and a trunk position of 0 is at the proximal base of the trunk and near the distal tip would be a value of 1.

Mathematical modeling of wrinkle function and surface area

The wrinkles along the trunk can be approximated as a sine wave with variable amplitude (A(z)) and wavelength ($\omega(z)$), with z as the distance from the tip. We will use the same wrinkle geometry calculations as previously computed by Schulz et. Al[18]. Therefore, the overall wrinkle function is:

$$f(z) = A(z)\sin(\omega(z))$$

Each elephant sample and region are analyzed with a given wavelength and frequency. Frequency is defined as the number of wrinkles in a unit step, for our case we are defining frequency as the number of wrinkles seen in a unit of length. Using this function, we can calculate surface area.

The surface area, *SA*, of elephant skin can be determined using the arclength of the specific sine function for both the dorsal and ventral portions of the trunk. We will denote arclength, *s*, as the length of a function *f*(*z*), which is a function of the distance from the distal tip of the trunk *z* given in meters. With a given elephant trunk of length, *L*, the arclength of the wrinkles can be computed as:

$$s = \int_{z=0}^{z=L} \sqrt{1 + \left(\frac{df}{dz}\right)^2}\, dx$$

the arclength integration is computed using the specific functions of *A*(*z*) and *ω*(*z*) input into f(z). The MATLAB *rsums* package to approximate the integration to determine the total arclength in meters.

We will split the trunk into different sections one to ten, as described previously. To calculate the surface area given the arclength *s* of a section between an initial trunk diameter d_0 and a final trunk diameter d_F would then be

$$SA = \frac{d_0 + d_F}{2} \cdot s$$

We use this equation to calculate the surface area of both the African and the Asian elephants and report the surface areas in the results.

Statistical analysis of wrinkle numbers

To determine the difference in numbers of major trunk wrinkles between adult Asian elephants (n = 7, out of these 5 females and 2 males) and adult African elephants (n = 7, all females) a two-sample t-test (Welch's t-test) was performed (Figure 2C). Adult Asian elephants ($\bar{x}$ = 126, SD = 25) have more major trunk wrinkles than adult African elephants ($\bar{x}$ = 83, SD = 13; two-sample t-test (12) = 4, p = 0.003, d = 2.16). There is no significant difference in minor wrinkles between the two species. When testing with numbers from only females a two-sample t-test (Pooled variance) revealed an even stronger difference between Asian (n = 5, $\bar{x}$ = 124, SD = 21) and African elephants (two-sample t-test (10) = 4, p = 0.002, d = 2.51). When looking at wrinkle numbers in different trunk zones (Figure S1C) no differences between species were found in the numbers of wrinkles on trunk shaft or tip, independently of pooling or not pooling major and minor wrinkles or female and male elephants. A two-sample t-test (Pooled variance) revealed a difference in major wrinkles on the trunk shaft, with adult Asian elephants (n = 7, out of these 5 females and 2 males) having more major trunk shaft wrinkles ($\bar{x}$ = 115, SD = 26) than adult African elephants (n = 7, $\bar{x}$ = 70, SD = 13; two-sample t-test (12) = 4, p = 0.002, d = 2.18). When again comparing only numbers of the female elephants the effect was slightly bigger (two-sample t-test (10) = 4, p = 0.002, d = 2.45). Additionally, when looking at a possible difference between dorsal and ventral wrinkles a two-tails paired t-test was performed (Figure 2F). The dorsal part of the trunk (M = 77, SD = 12) has significantly more wrinkles than the ventral part (M = 47, SD = 7; two-tails paired t-test (4) = 5.1, p = 0.007, d = 2.27).

To determine if the observed difference in wrinkles number between the longer whiskers side (n = 15) and the shorter side (n = 15) was significant a Wilcoxon Signed-Rank tests was performed (Figure 3B). It revealed that the difference was indeed significant (z-value = -2.1583, w-value = 22, p = 0.03078, d = -0.55727). A second Wilcoxon Signed-Rank tests revealed that there is no favored left or right preference in African and Asian elephant (z-value = -1.1927, w-value = 39, p = 0.23404, d = -0.30795) (Figure 3C).

Results

Asian and African elephants differ in their trunk folds and wrinkles

We studied trunk wrinkles in Asian (*Elephas maximus*) and African (*Loxodonta africana*) live elephants, in photographs and in post-mortem material. All of these sources indicated consistent differences between trunk wrinkles of African and Asian elephants. We show these differences in drawings and photographs of adult African and Asian elephant trunks (Figure 1). Asian elephants (Figure 1A) have more and proximally less prominent transversal trunk wrinkles and folds than African elephants (Figure 1B). Coloration of the trunk and skin texture differs between species, with Asian elephant trunk skin looking lighter, more pinkish, and smoother (Figure 1A Inset) and African elephant trunk skin appearing greyer and more cracked (Figure 1B Inset). In both species transversal wrinkles and folds are more clear and deeper than longitudinal trunk wrinkles (Figure 1) and there are few or no oblique (non-transversal or longitudinal) wrinkles. In the proximal half of the trunk, African elephants have relatively even spaced major wrinkles (folds) that look deep and in between shallow looking (minor) wrinkles. In the distal half the folds transition to densely packed major wrinkles that look similar in both species. It looks like in the proximal part of the trunk Asian elephants have more but less deep major wrinkles than African elephants. In both elephant species we also noticed numerous “broken” wrinkles, transversing around half of the trunk from one side and after a gap often continuing shifted a little bit proximally or distally. It seems like Asian elephants also have several major wrinkles that look deeper than others and are appearing repeatedly along the trunk. The remainder of our paper will focus on transversal wrinkles and folds. We conclude that Asian and African elephants have visually distinct patterns of trunk wrinkles and folds.

Transversal trunk folds and wrinkles differ in counts and distribution between Asian and African elephants

Wrinkles were traced on photographs of elephant trunks and color coded according to wrinkle type, which could be major or minor wrinkles (Figure 2A and B). Adult Asian elephants (n = 7) have significantly more dorsal major trunk wrinkles ($\bar{x}$ = 126, SD = 25) than African elephants (n = 7, $\bar{x}$ = 83, SD = 13; two-sample t(12) = 4, p = 0.003). Asian elephants have more minor wrinkles in the proximal part of the trunk and more major wrinkles throughout the whole trunk, whereas African elephants have more regularly spaced major and minor wrinkles in the proximal part and more minor wrinkles in the distal part of the trunk (Figure 2D and E). In both species (n = 5) we found significantly more major wrinkles dorsally (M = 77, SD = 12) than ventrally (M = 47, SD = 7; two-tails paired t-test(4) = 5.1, p = 0.007, d = 2.27; Figure 2F). For a more detailed analysis we compared numbers of minor and major wrinkles in Asian and African elephant calves and adults according to trunk zones, which can be base, shaft or tip of the trunk (Figure S1A and B). In calves of both species (Asian elephant calves base and tip n = 3, shaft n = 4; African elephant calves all trunk zones n = 2), we found comparable numbers of major and minor wrinkles, but Asian elephants gain more wrinkles during their life than African elephants (Figure S1C). The differences in wrinkle numbers between the two species are reflecting differences in number of major wrinkles, but not minor wrinkles (Figure 2C and figure S1C). More specifically we see these differences only in the shaft of the trunk and not in the tip or base, with adult Asian elephants (base and shaft n = 7, tip n = 10) having more major trunk shaft wrinkles ($\bar{x}$ = 115, SD = 26) than adult African elephants (base and shaft n = 7, tip n = 9; $\bar{x}$ = 70, SD = 13; two-sample t-test(12) = 4, p = 0.001, d = 2.18; Figure S1C).

Trunk wrinkle number is lateralized

Almost all adult elephants show marked left-right asymmetries in whisker length as shown for the trunk tip of African elephant Indra on Figure 3A. Whisker were longer on Indra's right trunk side; thus she presumably was a right-trunker preferentially grasping/wrapping towards her right side. We found slightly more wrinkles on her right trunk tip side than on her left trunk tip side (20; Figure 3A). This bias of having more wrinkles on the trunk side with longer whiskers was systematic (Figure 3B) and significant (Wilcoxon Signed-Rank, z-value = -2.1583, w-value = 22, p = 0.03078, d = -0.55727) in our African (n = 5) and Asian (n = 10) elephants. We observed 10% less wrinkles than on the longer whiskers side (Figure 3B). In contrast we did not observe a systematic difference in trunk side wrinkle numbers as a function of left vs right trunk side (Figure 3C) (Wilcoxon Signed-Rank test, z-value = -1.1927, w-value = 39, p = 0.23404, d = -0.30795), nor with a species difference (Figure 3B-C). The absence of such a difference is in line with the fact that elephants do not show an overall trunk lateralization bias towards the right or left side. We had one case of an ambidextrous African elephant with no whisker or wrinkle difference, reinforcing our theory of the modification of the wrinkle pattern based on a user dependant experience. We conclude that elephant trunk wrinkle number is larger on the trunk side with longer whiskers, i.e. the side towards which elephants grasp/wrap. Because trunk lateralization emerges with functional ability of trunk, which takes nearly two years, our data also indicate the trunk wrinkle patterns are affected by use/ experience.

Visualization of trunk wrinkles in microCT scans

To visualize trunk wrinkles and underlying skin structure we obtained microCT scans of iodine stained trunks of Asian and African elephant calves/fetuses. Such scans provided high-resolution images of entire elephant trunks (Figure 4). Wrinkles and folds were readily visible in volume renderings (Figure 4A) and parasagittal sections (Figure 4B) of an Asian baby elephant trunk. As we noted before in adult trunks, wrinkle frequency increased from proximal to distal (Figure 4A-D). Remarkably, it appears that the morphology of the different skin layers differs on the trough of a fold compared to that of the fold's peak (Figure 4C-D). Thus, trunk folds are not mere folds of the skin, but they go along with major changes in skin structure. In particular, because of the lack of inner skin elements, the skin is quite thin within folds and presumably also more flexible than in between folds. Very similar observations were made on volume renderings (Figure 4E) and parasagittal sections (Figure 4F) of an African baby elephant trunk. Similar to the Asian baby elephant, the outer skin of the African baby elephant was observed to be of constant thickness throughout folds, while we found inner skin to be relatively thicker between folds than in folds (Figure 4G). We conclude that outer and inner elephant skin differ markedly in their skin fold and wrinkle pattern.

Wrinkle and amplitude differentiation in microCT

The African baby elephant had larger wavelengths between each of the wrinkles compared to that of the Asian elephant calf. From the base to the tip the African elephant foetus trunk had a steady decrease in distance between the wrinkles, whereas the distal half of the Asian baby elephant having nearly constant wavelength (Figure 5A). In viewing the amplitude or depth of each of the wrinkles both African and Asian elephant babies had increasing amplitude approaching the base, however the Asian elephant calf had deeper amplitudes all along the trunk (Figure 5B). With utilizing the amplitude and wavelength of the wrinkles we can characterize a sinusoidal fit of the wrinkles and folds to determine an approximate surface length of each the elephant species (Figure 5C). From the data we see that Asian baby elephant wrinkles occur more frequently (smaller wavelengths) and are deeper (more amplitude). This leads to Asian baby elephants having a much larger surface as they are deeper and closer together leading to increased overall surface length (Figure 5C).

Fetal trunk and trunk wrinkle development

We wondered how trunk wrinkles develop and how their development relates to trunk development in general. To address this issue, we studied fetal elephant specimens (n = 3 African elephant fetuses, n = 2 Asian elephant fetuses) and published photographs or drawings of elephant fetuses (n = 50 African, n = 12 Asian). We then assigned embryonic ages to these specimen as detailed in the methods section.

Figure 6A shows schematic drawings of different stages of fetal African elephant heads, their trunks (black), their trunk wrinkles (gray), their upper (red) and lower (green) lip. The elephant trunk develops from a large and probably already wrinkled nose primordium. Wrinkle are added initially rapidly and then more gradually. A schematic overview is given for wrinkle development (Figure 6B) and lip development (Figure 6C). The upper lip fusion occurs rapidly between embryonic day 100 and 130.

Relative to early fetuses the trunk shows more length growth than other elephant body parts; this faster than other body parts trunk growth occurs early (E60 -E150, Figure 6D) The fetal trunk length growth pattern is similar in African and Asian elephants (Figure 6E). A log-plot of wrinkle number against fetal age reveals that wrinkle develop in two sharply different phases (Figure 6F). Between E60 and E150 there is an exponential growth of wrinkle number with a doubling time of about 20 days. After that addition of wrinkles is slow, and slightly faster in Asian elephant fetuses than in African ones.

Figure 7A gives a schematic overview for trunk tip development. The trunk first grows as a stump. Then around embryonic day 130 the ventral finger grows out in African elephants, whereas Asians grow out a bulbous ventral trunk tip structure. The fact that the ventral finger grows out first is shown in Figure 7B for African elephants. Specifically, we observed that the ventral finger tends to longer between E120 (before that there are no fingers) and E200. Dorsal finger development follows slight delayed in both species and differs in time course between Asian and African elephants. In African elephants, finger growth goes through a brief initial exponential length increase, after which finger is slower and more gradual (Figure 7C), our data were insufficient for a detailed assessment finger growth patterns in Asian elephants.
We conclude that the trunk in the fastest growing body part of elephants and that wrinkles are added in two steps, a first exponential growth step, and second slower addition step, which differs between Asian and African elephants.

Discussion

We assessed elephant trunk wrinkle and their development by photography and microCT imaging in a substantial sample of fetal, newborn and adult African and Asian elephants. Asian elephants had significantly more dorsal trunk wrinkles (~126) than African savanna elephants (~83). In both species, we find more dorsal than ventral trunk wrinkles and a closer spacing of wrinkles in the distal than in the proximal trunk. MicroCT imaging revealed the skin structure of folds. In both Asian and African elephant fetuses, trunk length grows more than the length of other elephant body parts. In development, trunk wrinkles are added in two distinct phases, an early exponential phase and a later phase, where wrinkles are added slowly. The idea that wrinkles improve the ability of trunk skin to bend might explain many of our findings, in particular the dorsoventral, proximal-distal, lateralized and species differences in wrinkle distribution.

Asian elephants have more trunk wrinkles than African elephants

We find the trunk wrinkles of African and Asian elephants to be remarkably different (Figures 1 and 2). Specifically, Asian elephants have about 1.5 times more trunk wrinkles than African elephants. We are surprised that this rather striking species difference in trunk morphology found relatively little attention so far. What contributed to the detection of this difference is the relatively large sample of histological trunk samples, which reflect the several decade collection efforts of the Institute of Zoo and Wildlife research, Berlin (IZW). Our analysis extends earlier work on the folding / wrinkle structure of elephant skin[17].

Trunk bending flexibility might explain wrinkle distribution patterns

We found elaborate patterns of trunk wrinkles. There are more wrinkles on the dorsal than on the ventral trunk, there are more closely spaced wrinkles on the distal than on the proximal trunk and as discussed above there are more trunk wrinkles in Asian than in African elephants. It should be noted that a majority of trunk manipulation movements are accomplished with gripping and grabbing on the ventral side[48]. The distal tip of the trunk has large volumes of oblique and transverse muscles and it utilized more often for fine motor tasks[17,48–50]. We note that inward bending of the trunk (i.e. sticking the trunk tip into the mouth) is seen in elephants much more often than outward bending of the trunk. As a result, the dorsal side of the trunk experiences more curvature over time and specifically the distal tip experiences more wrapping and curvature patterns. Similarly, the distal trunk is much more flexible than the proximal trunk in elephants and bending is more common distally than proximally. Finally, Asian elephants tend to bend their trunk more than African elephants, because they pursue a grasping/trunk wrapping strategy for object handling as opposed to the pinching strategy of African elephants[12]. A synopsis of these findings leads a simple, biomechanically plausible explanation of trunk wrinkle distribution that at birth elephants are born with wrinkles and through age and development. Additionally, the elephant has the means to add wrinkles due to mechanical stressors experienced in their daily manipulation tasks. This is seen in the increased presence of distal tip wrinkles, but also in their lateralization.

Lateralization leads to wrinkling

Elephant trunks exhibit lateralization, or handedness, with their trunks[9]. The muscle morphology of a trunk includes oblique muscles located at the distal tip ventral portion and elephants will prefer a direction when executing complex motion tasks. This lateralization has been shown in both African[10] and Asian elephants[9]. Lateralization additionally leads to morphological changes outside of the muscle with abrasions lead to shorter whiskers and hairs[11]. Additionally, this lateralization means the trunk is contacting the ground more often with the preferred side causing additional force and abrasions at the distal tip. It appears from our results that along with development of additional wrinkles form during aging and that elephants also develop wrinkles on their dominant side from functional usage. Lateralization in elephants indicates that they curve to wrap and pick up objects to a specific side, left or right[49]. As discussed previously the elephant when manipulating objects pivots about a pseudo-joint, which wrinkles assist in this extension. As elephants are stretching towards their dominant side, it is possible that through stress on the skin of constant stretching causing development of additional wrinkles on the non-dominant side.

Tip lateralization stressors from stretching lead to increased wrinkles on the opposed lateral side, and additional wrapping towards the ventral section leads to increased wrinkles on the dorsal trunk. These wrinkles are developed in a targeted area along the side of the trunk and could provide mechanical benefits in gripping or extension as the wrinkles on the dorsal and ventral side have been shown[17,18].

Structure of folds and wrinkles

Overall the two species studied exhibit vastly different wrinkle number as well as distance between wrinkles both at birth and after development. As the elephants grow their trunks increase in size, length, muscle mass. As we see that African elephant (*Loxodonta Africana)* are larger when compared to Asian elephants (*Elephas maximus)* it is initially surprising that they exhibit less wrinkles, however in studying the morphology of their trunks African elephants have thick folds that can be several cm in distance from each other[17]. Whereas when we view the Asian elephant trunk we see at the proximal folds have several wrinkles in-between. With African elephants exhibiting much less folds and larger trunks it is simple to see that the wrinkles and folds on the elephant trunk are likely becoming deeper with larger amplitudes. There could also be temperate factors in the difference of the African versus Asian wrinkles and folds as African elephants are in environments with less humidity and wrinkling on the skin has been shown to have thermoregulation properties[15,51]. Wrinkles are often seen as instabilities developed through stress or force, but the elephant has very unique wrinkles as they are present days into their pre-natal development.

Trunk and trunk wrinkle development

We analyzed elephant trunk development and trunk wrinkle development in fetal specimens and photographs of elephant fetuses. We find that the trunk shows exceptional growth in early pregnancy (E60-E150) that exceeds that of other body parts. These findings align with earlier conclusions on trunk development from transrectal ultrasound imaging[36]. Even the earliest elephant (~E60) fetuses, for which photographs are available have a large nose with periodic irregularities, possible precursors of wrinkles. With respect to wrinkles our analysis indicates a bipartite developmental pattern. First, we observe an exponential increase of wrinkle number with a doubling of wrinkle number approximately every 20 days; this period coincides with the time of very fast trunk extension. Then, at ~E130 the increase in wrinkle number slows down sharply and continues to be slow into adulthood. Our observations also provide a staging/ dating of the upper lip nose fusion[32,33], which appears to occur between postnatal E100 and E130, i.e. roughly in the fourth month of pregnancy. Finally, we find that trunk tip development trails trunk extension and that the trunk first grows out as a stump. Then, after E120 the fingers develop, whereby the ventral finger extends first. The bulbous ventral trunk tip of Asian elephants is present at an early time point ~E130. This early appearance of bulbous ventral trunk tip in Asian elephants might suggest that the differential trunk tip specialization was an early event in the species differentiation of African and Asian elephants.

Conclusion

We suggest trunk wrinkles and their unique molding in African and Asian elephants contribute to the phenomenally flexible actuation of elephant trunks. Elephants develop their wrinkled trunks both pre and post birth. After an early wrinkle (E60-E130) addition both elephant species elephants grow to a plateau of around 70 wrinkles. African elephants increase less in wrinkle number. We see elephants have functional wrinkle development through trunk use. Specifically, elephants bend more with their dorsal side to grip objects and wrap leading to additional wrinkles not just on the dorsal side, but also on the lateral side, caused by the lateralization in the trunk. We conclude that elephants develop their wrinkles through their form as a means of growth, both pre and post birth.

Data Access Statement

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data reported in this paper is shared on a publicly accessible repository. This paper does not report the original code.

Ethics Statement

All material and morphology measurements were taken from photos of previously dissected species. Specific information about each of the individuals analyzed is included in the supplement.

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Conflict of Interest Statement

The authors declare no conflicts of interest in any of this manuscript.

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Figures

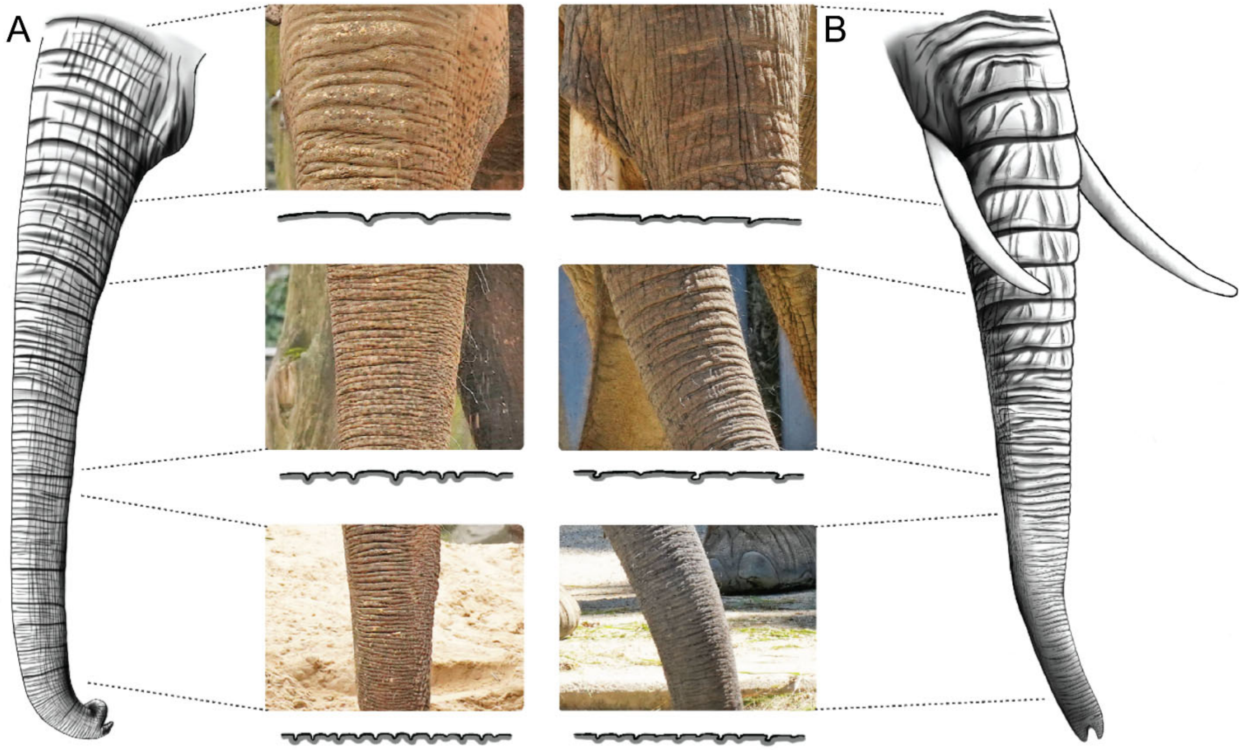

Figure 1: Asian and African elephants differ in their trunk folds and wrinkles

A, Drawing of the trunk of an adult Asian (*Elephas maximus*) elephant. Note the larger number of transversal wrinkles in the Asian elephant compared to the African elephant (*Loxodonta africana*) trunk in **B**. Insets are displaying the proximal base wrinkles (top) to the distal tip wrinkles (bottom).

B, Drawing of the trunk of an adult African elephant with insets displaying base folds of an elephant (top) to the distal tip wrinkles.

Photo credit (A): Lena Kaufmann, Humboldt Universität zu Berlin; Zoologischer Garten Berlin, Berlin, Germany.
Photo credit (B): Lena Kaufmann, Humboldt Universität zu Berlin; Zoo Schönbrunn, Vienna, Austria.

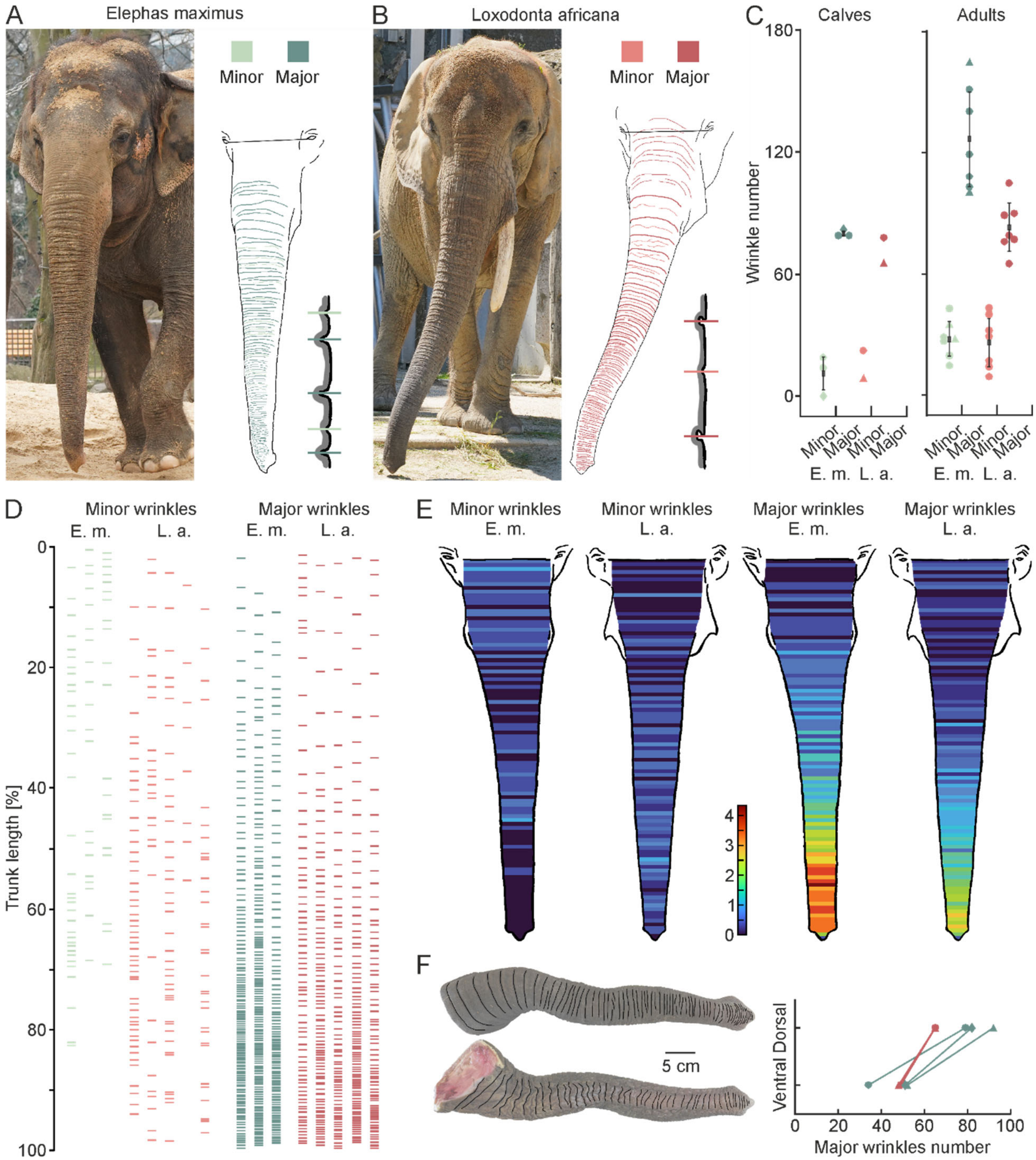

Figure 2: Transversal trunk folds and wrinkles differ in counts and distribution between Asian and African elephants

A, Female Asian elephant Carla (Zoo Berlin) next to a tracing of minor and major wrinkles with a schematic of what minor/major wrinkles look like in Asian elephants. Note the zero-line between the eyes, wrinkles proximal to this were not included in the analysis.

B, Same as A but for female African elephant Drumbo (at time of photograph Zoo Schönbrunn).

C, Comparison of trunk wrinkle numbers between Asian and African elephants in calves (left) and adults (right). Asian elephant calves (n = 3) and African elephant calves (n = 2) have similar numbers of minor and major wrinkles. Asian adult elephants (n = 7) have more major trunk wrinkles than African adult (n = 7) elephants (two-sample t(12) = 4, p = 0.003, d = 2.16). Circle = female, triangle = male, diamond = unknown.

D, Positions of minor (left) and major (right) wrinkles on trunks normalized to total trunk length in individual female Asian (n = 3) and African (n= 5) elephants.

E, Heatmaps showing the distribution of minor (left) and major (right) trunk wrinkles in Asian (n = 3) and African (n = 5) elephants. Asian elephants have on average more minor wrinkles in the proximal part of the trunk whereas in African elephants minor wrinkles are more spread over the rest of the trunk. Asian elephants have on average more major wrinkles in the distal half of the trunk with a particularly high density in the region they bend when wrapping objects. Blue is showing a low average density of wrinkles and red a high average density of wrinkles at this position of the trunk.

F, On the left a photograph of an African elephant calf trunk showing tracings of major wrinkles on the dorsal (upper) and ventral (lower) sides of the trunk. On the right a comparison of dorsal and ventral major trunk wrinkles in Asian (n = 3) and African (n = 2) elephants, there are significantly more wrinkles on the dorsal (M = 77, SD = 12) than on the ventral side of the trunk (M = 47, SD = 7; two-tails paired t-test(4) = 5.1, p = 0.007, d = 2.27)

Photo credit (A): Lena Kaufmann, Humboldt Universität zu Berlin; Zoologischer Garten Berlin, Berlin, Germany.
Photo credit (B): Lena Kaufmann, Humboldt Universität zu Berlin; Zoo Schönbrunn, Vienna, Austria.
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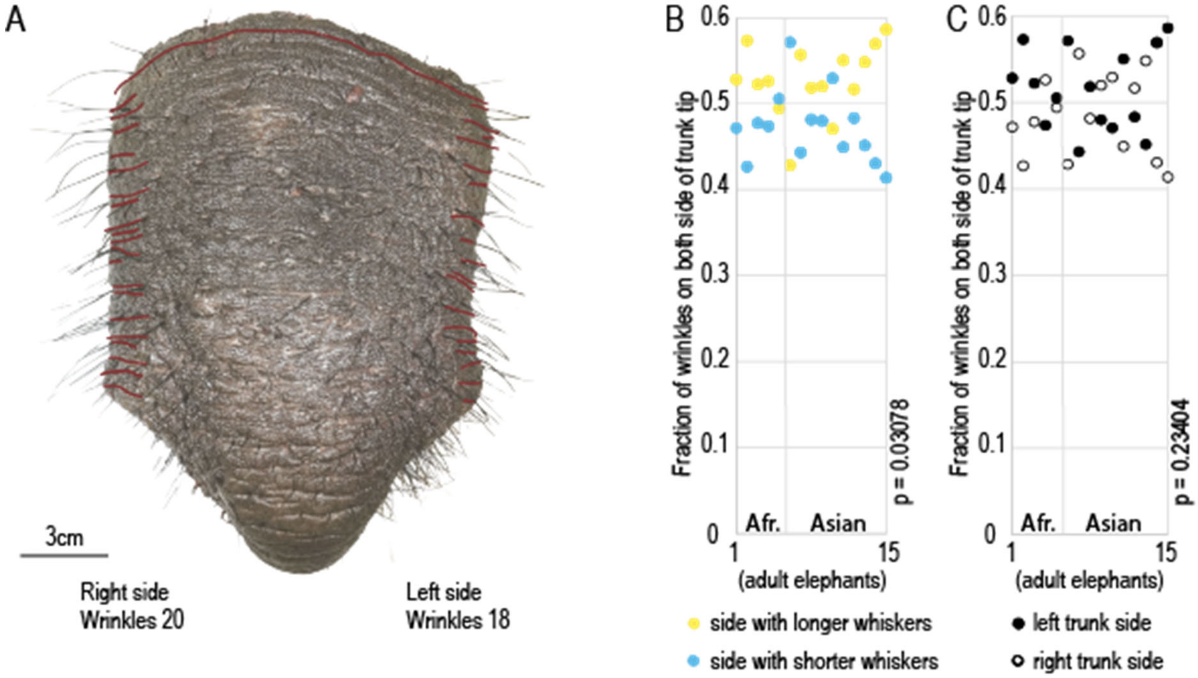

Figure 3. Left- and right-trunkers have more wrinkles on the left and right trunk side, respectively

A, African Elephant trunk tip, in red the fold/major wrinkles. The red line crossing the tip is the last wrinkle count.

B, Univariate plot of the fraction of wrinkles (normalized to the total count on both trunk sides) on the trunk side with shorter or longer whiskers. Trunk function is lateralized in elephants and so-called left-trunkers, who preferentially grasp towards the left side, have shorter whiskers of the right trunk side[11]; the reverse is true for right-trunkers. We observed ~10% more wrinkles on the longer whisker side. In yellow the wrinkles fraction on the longer whisker side and in blue: wrinkles on the shorter whisker side. One dot is one animal, yellow and blue for the same animal plotted in the same axis. The wrinkles counts are normalized by the total wrinkles count. (Wilcoxon test, z-value = -2.1583, w-value = 22, $p = 0.03078$, d = -0.55727) (5 adult African elephants, 9 adult Asian elephants).

C, Univariate plot of the fraction of wrinkles (normalized to the total count on both trunk sides) on the left or right side of the trunk. The full dots are the wrinkles fraction on the left side and the empty dots the wrinkles on the right side. One dot is one animal, for the same animal the dots are plotted in the same axis. The wrinkles counts are normalized by the total wrinkles count. (Wilcoxon test, z-value = -1.1927, w-value = 39, $p = 0.23404$, d = -0.30795) (5 adult African elephants, 9 adult Asian elephants).

Photo credit (A): Lena Kaufmann, Humboldt Universität zu Berlin.

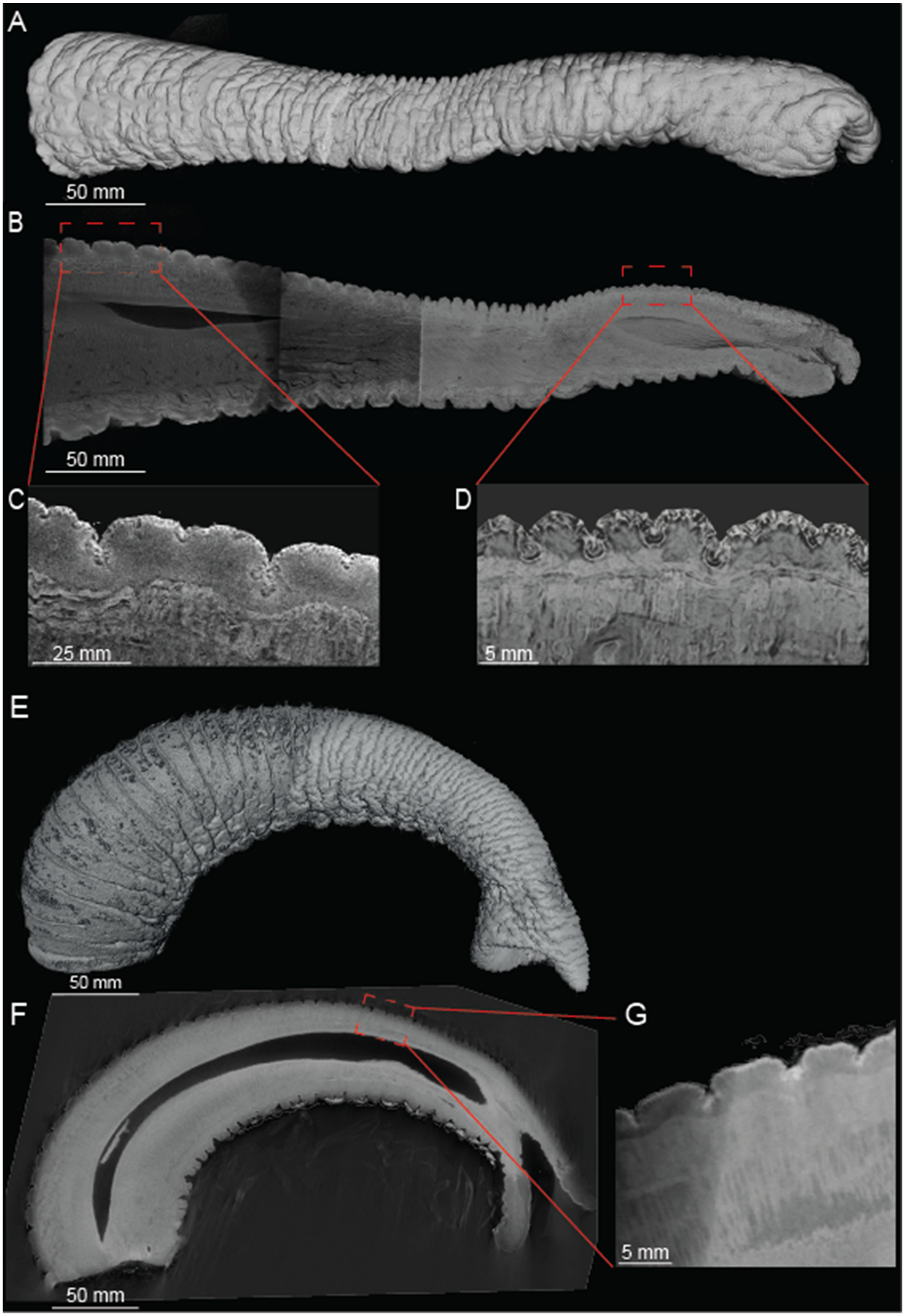
A
50 mm
B
50 mm
C
25 mm
D
5 mm
E
50 mm
F
50 mm
G
5 mm

Figure 4. Visualization of trunk wrinkles in microCT scans of an Asian and an African baby elephant trunk

A, Volume rendering of a µCT scanned Asian baby elephant trunk.

B, Sagittal slice of an Asian baby elephant trunk.

C, High magnification view of proximal dorsal wrinkles in the Asian baby elephant trunk. Note the constant thickness of the outer skin and the relatively thicker inner skin between folds compared to the inner skin below folds.

D, High magnification view of distal dorsal wrinkles in the Asian baby elephant trunk.

E, Volume rendering of a µCT scanned African baby elephant trunk.

F, Sagittal slice of an African baby elephant trunk

G, High magnification view of dorsal wrinkles in the African baby elephant trunk. Note the constant thickness of the outer skin and the relatively thicker inner skin between folds compared to the inner skin below folds.

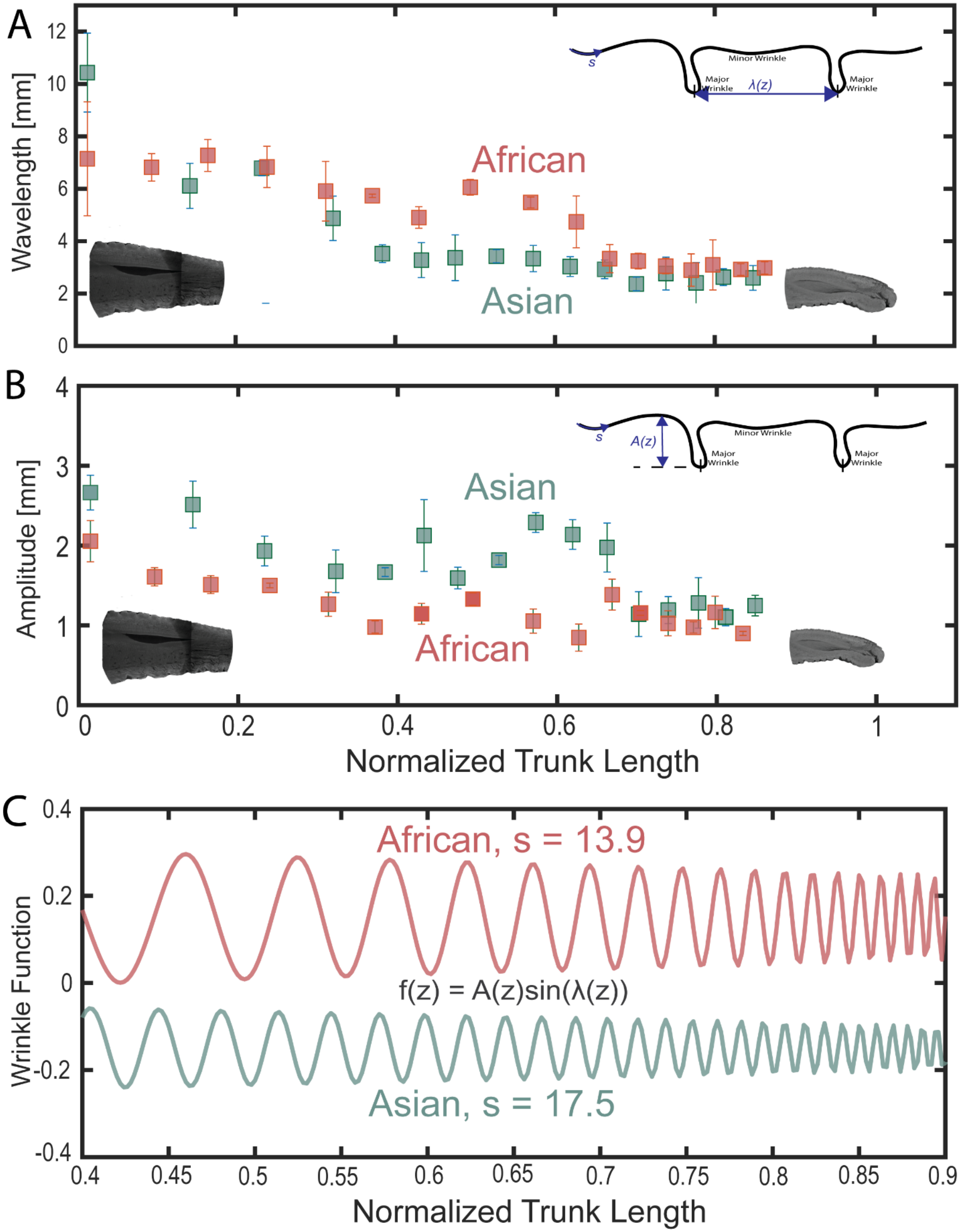
A
Wavelength [mm]
Minor Wrinkle
Major Wrinkle
λ(z)
s
African
Asian
B
Amplitude [mm]
A(z)
Asian
African
Normalized Trunk Length
C
Wrinkle Function
African, s = 13.9
f(z) = A(z)sin(λ(z))
Asian, s = 17.5
Normalized Trunk Length

Figure 5 Wrinkle amplitude, wavelength, and function between African and Asian elephants:

A, Wavelength of African Elephants (orange) and Asian elephants (green) of baby elephants taken from microCT in Figure 3.

B, Peak-to-peak amplitude of African Elephants (orange) and Asian elephants (green) of baby elephants taken from microCT in Figure 3.

C, Plotted function of what the trunks amplitude and wavelength are along with the total length of a normalized trunk. Interpolated sine waves from the wavelength and amplitude data in A-B showing the functional difference between African (orange) and Asian (green) elephant trunks.

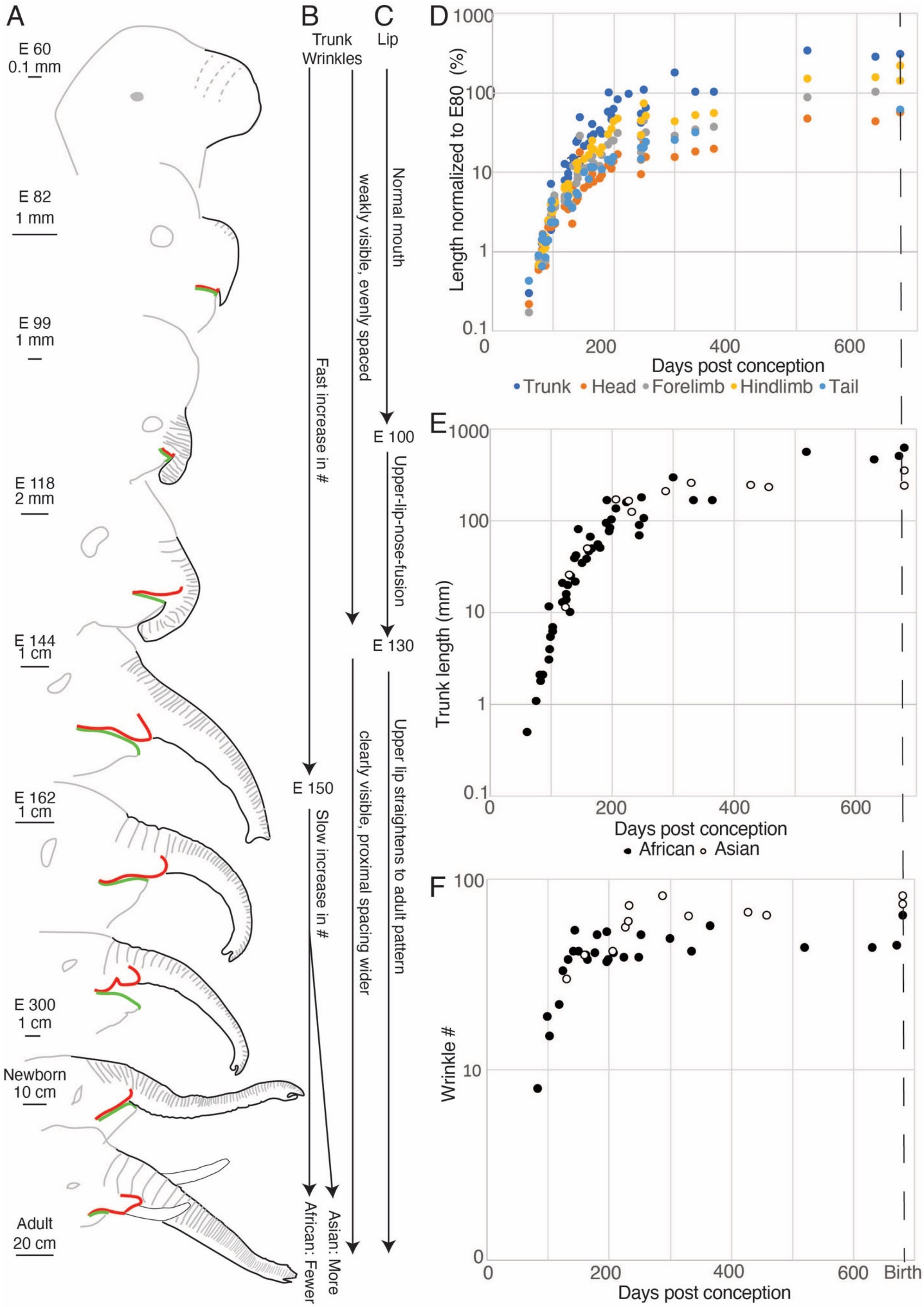
A
E 60
0.1 mm
E 82
1 mm
E 99
1 mm
E 118
2 mm
E 144
1 cm
E 162
1 cm
E 300
1 cm
Newborn
10 cm
Adult
20 cm
B
Trunk
Wrinkles
Fast increase in #
weakly visible, evenly spaced
E 150
Slow increase in #
clearly visible, proximal spacing wider
African: Fewer
Asian: More
C
Lip
Normal mouth
E 100
Upper-lip-nose-fusion
E 130
Upper lip straightens to adult pattern
D
Length normalized to E80 (%)
Days post conception
Trunk
Head
Forelimb
Hindlimb
Tail
E
Trunk length (mm)
Days post conception
African
Asian
F
Wrinkle #
Days post conception
Birth

Figure 6: Fetal trunk and trunk wrinkle development

A, Schematic drawings of African elephant fetuses. Gray, outline of the head and wrinkles; gray dashed, putative wrinkles; black, outline of the trunk; green, lower lip; red, upper lip. Fetuses were redrawn from references following references[13,22–47].

B, Schematic of stages of fetal wrinkle development in African and Asian elephants.

C, Schematic of stages of fetal lip development in African elephants.

D, Relative length growth of various body parts in African elephants. Length was normalized to the length of the respective body part in E80 fetuses and is given in %. The trunk grows more than other body parts and the accelerated growth occurs mainly between E60 and E150.

E, trunk length growth in African and Asian elephants is similar.

F, wrinkle development African and Asian elephants. Wrinkle number increases in sharply different phases: Between E80 and E130 there is an exponential increase in wrinkle number with a doubling time of ~ 20 days. After E130 addition of wrinkles is slow, but slightly faster in Asian than in African elephants.

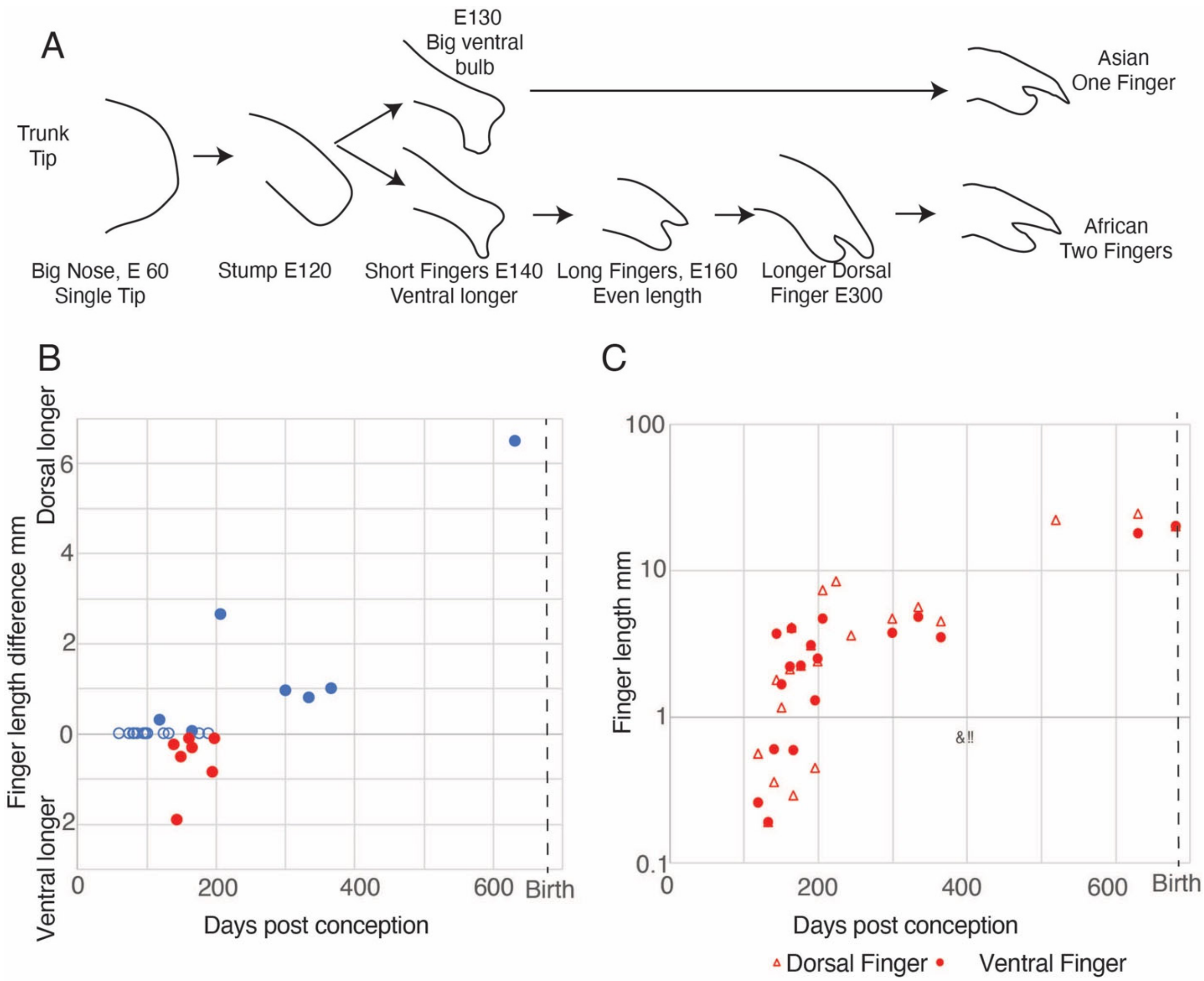

Figure 7: Fetal trunk finger development in Asian and African elephants

A, Schematic of stages of trunk tip development in African and Asian elephants.

B, Length difference of dorsal and ventral trunk finger in African elephants over the course of fetal development. Pre E120 there are no fingers, then the ventral finger is longer (highlighted as red dots) and after E200 the dorsal finger takes over.

C, Fetal dorsal and ventral finger growth in African elephants. Finger growth goes through a brief exponential phase (E130-E180), after which finger growth slows down. The length of both the dorsal and ventral finger could not be determined in all specimen. Note that the ventral finger (circles) tends to be longer than the dorsal finger (triangles) in early fetuses and shorter than the dorsal finger in older fetuses.

Supplemental Figures:

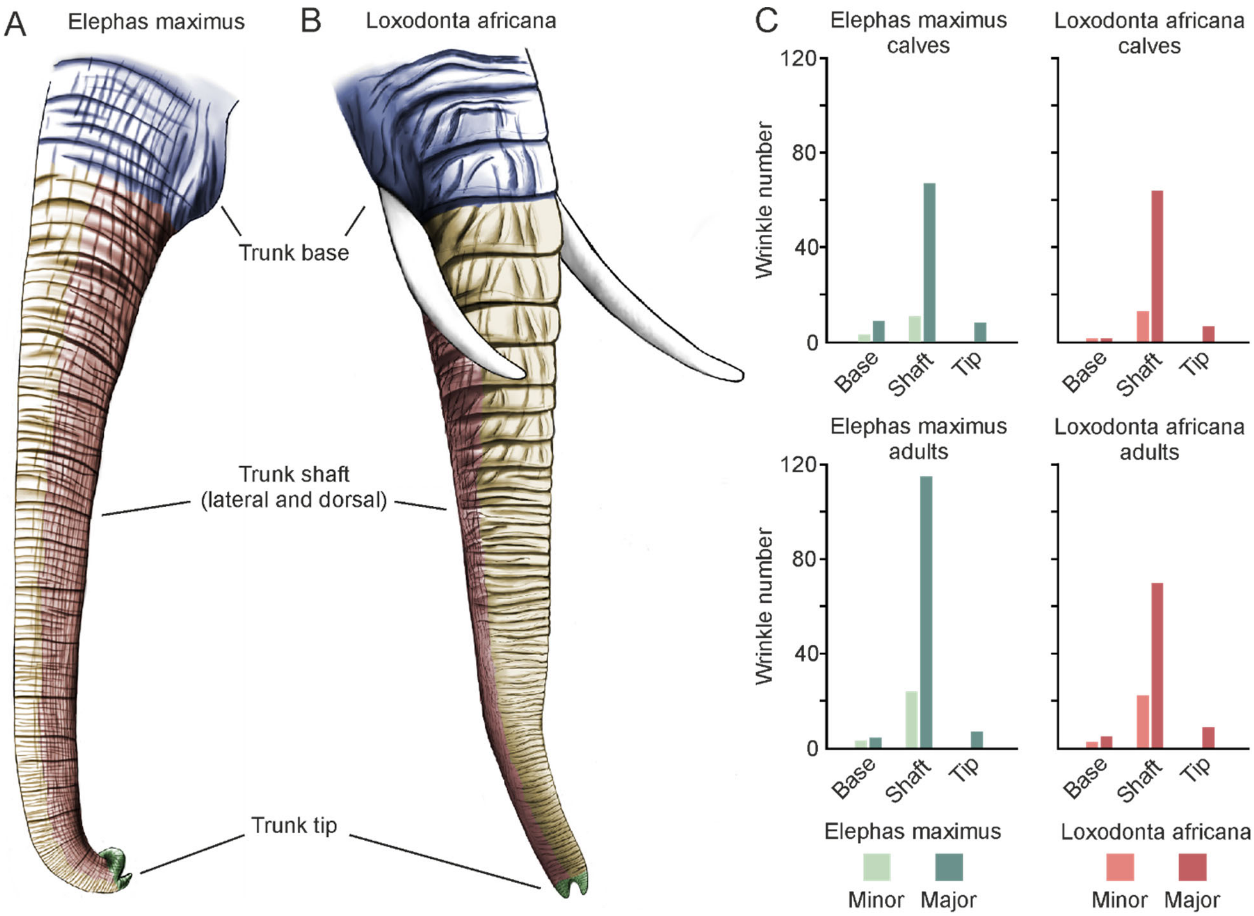

Figure S1: Differences of trunk wrinkle numbers between Asian and African elephants according to trunk zones and as a result of postnatal ontogeny

A, Drawing of an Asian elephant (*Elephas maximus*) trunk, separated in three distinct zones, base, shaft (lateral and dorsal), and tip of the trunk.

B, Same as in **A** but for an African elephant (*Loxodonta africana*) trunk.

C, Minor and major wrinkle numbers in Asian (left) and African (right) elephant calves (top) and adults (bottom). Asian elephant calves (base and tip n = 3, shaft n = 4) and African elephant calves (all trunk zones n = 2) have similar numbers of both minor and major wrinkles. Adult Asian elephants (base and shaft n = 7, tip n = 10) and adult African elephants (base and shaft n = 7, tip n = 9) have comparable numbers of minor wrinkles in all trunk zones and of major wrinkles in base and tip of the trunk. Adult Asian elephants have on average more major wrinkles in the shaft of the trunk ($\bar{x}$ = 115, SD = 26) than adult African elephants ($\bar{x}$ = 70, SD = 13; two-sample t-test (12) = 4, p = 0.001, d = 2.18).

Table S1: Overview elephant lab samples, treatment of specimen and derived data

Name (*species*)	**Sex**	**Location at death**	**Age (y), died on (dd/mm/yy)**	**Specimen treatment**	**Data derived**
Ilona (*E. maximus*)	F	Zoo Karlsruhe, Germany	~45, 31/03/14	Frozen for several years, in fixative (4% PFA)	Wrinkle numbers trunk tip
Vilja (*E. maximus*)	F	Wilhelma Stuttgart, Germany	~61, 10/07/10	Frozen for several years	Wrinkle numbers trunk tip
Naing Thein (*E. maximus*)	M	Zoo Kopenhagen, Denmark	40, 10/02/21	In fixative (4% PFA)	Wrinkle numbers trunk tip
Asian Baby (*E. maximus*)	Unknown	Tierpark Hagenbeck, Germany	0, 2012	Frozen for several years, in fixative (4% PFA)	Wrinkle numbers trunk tip, shaft and base
Hoa's Baby (*E. maximus*)	F	Zoo Leipzig, Germany	0, 2015	Frozen for several years, in fixative (4% PFA)	Wrinkle numbers trunk tip, shaft and most of base
Indra (*L. africana*)	F	Elefantenhof Platschow, Germany	~35, 06/09/22	In fixative (4% PFA)	Wrinkles numbers trunk tip, shaft, and base
Linda (*L. africana*)	F	Zoo Poznan, Poland	~35, 02/02/21	Frozen for several years	Wrinkle numbers trunk tip
Zimba (*L. africana*)	F	Opel-Zoo Kronberg, Germany	~39, 10/04/21	In fixative (4% PFA)	Wrinkle numbers trunk tip
Ali (*L. africana*)	M	Opel-Zoo Kronberg, Germany	~24, 19/01/04	Frozen for several years	Wrinkle numbers trunk tip
AM 1 (*L. africana*)	M	Colchester Zoo, Great Britain	0, 2011	Frozen for several years, in fixative (4% PFA), iodine stained and destained for CT scanning	Wrinkle numbers trunk tip, shaft and base

Table S2: Overview zoo elephants, photographs used and derived data

Name (*species*)	**Sex**	**Location at time of photographs**	**Age at time of photographs**	**Data derived**
Anchali (*E. maximus*)	F	Zoo Berlin, Germany	3 d; 10 y	Wrinkle numbers trunk tip, shaft, and base for baby and adult
Carla (*E. maximus*)	F	Zoo Berlin, Germany	~49 y	Wrinkle numbers trunk tip, shaft, and base
Drumbo / Mary (*E. maximus*)	F	Zoo Berlin, Germany	~53 y	Wrinkle numbers trunk tip, shaft, and base
Malka (*E. maximus*)	F	Zoo Tbilisi, Georgia	~26 y	Wrinkle numbers trunk tip, shaft, and base
Pang Pha (*E. maximus*)	F	Zoo Berlin, Germany	36 y	Wrinkle numbers trunk tip, shaft, and base
Elbrus / Grand (*E. maximus*)	M	Zoo Tbilisi, Georgia	27 y	Wrinkle numbers trunk tip, shaft, and base
Victor (*E. maximus*)	M	Zoo Berlin, Germany	29 y	Wrinkle numbers trunk tip, shaft, and base
Hoa's Baby (*E. maximus*)	F	Zoo Leipzig, Germany	6 days old	Wrinkle number trunk base
Drumbo (*L. africana*)	F	Zoo Schönbrunn, Austria	~46 y	Wrinkle numbers trunk tip, shaft, and base
Iqhwa (*L. africana*)	F	Zoo Schönbrunn, Austria	9 y	Wrinkle numbers trunk tip, shaft, and base
Linda (*L. africana*)	F	Zoo Poznan, Poland	~26 y	Wrinkle numbers trunk tip, shaft, and base
Mongu (*L. africana*)	F	Zoo Schönbrunn, Austria	19 y	Wrinkle numbers trunk tip, shaft, and base
Numbi (*L. africana*)	F	Zoo Schönbrunn, Austria	~28 y	Wrinkle numbers trunk tip, shaft, and base
Tonga (*L. africana*)	F	Zoo Schönbrunn, Austria	~38 y	Wrinkle numbers trunk tip, shaft, and base
Kibali (*L. africana*)	F	Zoo Schönbrunn, Austria	1 y	Wrinkle numbers trunk tip, shaft, and base