Review History

RSOS-201126.R0 (Original submission)

Review form: Reviewer 1 (Enrique Peñalver)

Is the manuscript scientifically sound in its present form?
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Yes

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Have you any concerns about statistical analyses in this paper?
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Comments to the Author(s)
I included some corrections and comments in the pdf file of the manuscript (Appendix A).

Review form: Reviewer 2

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Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
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Have you any concerns about statistical analyses in this paper?
Yes

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Comments to the Author(s)
RSOS-201126

GEOMETRIC MORPHOMETRICS OF ENDOPHYTIC OVIPosition TRACES OF ODONATA (EOCENE, ARGENTINA)

The authors have attempted to study the shape variation of the egg traces of Odonata from the Eocene in Argentina, the article sound very original and the use of elliptical analyses of Fourier are indeed interesting, I missed a more sophisticated analysis implemented in the package “Momocs” for Fourier analyses, the use of old implementation is not bad but always can be better.

I have some comments written in the manuscript PDF (Appendix B) please take a look all of them mostly are of form, but I have some principal concern regarding the substrate variation, the authors said very explicit that there are no pattern related, but I noticed at least 3 pattern of 3 different substrate in the PCA, from Eucalyptus chubutensis (very clear variation in mostly all the morphospace) and in Malvaceae (very narrowed group of specimens not superposed to the others).

Nevertheless, I like the idea and I’m sure that the article can be accepted after minor revision comments of the PDF and improve the manuscript

Review form: Reviewer 3

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes
Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
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Comments to the Author(s)
Dear Authors,

Congrats for your manuscript. There is tiny mistakes to be corrected with english and one sentence in the discussion that seems awkward to me.

Otherwise, manuscript deserves to be publish as is.

Illustration and the effort of represent each oviposition per leaf in a draw are very good.

Decision letter (RSOS-201126.R0)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Romero-Lebrón

On behalf of the Editors, we are pleased to inform you that your Manuscript RSOS-201126 "GEOMETRIC MORPHOMETRICS OF ENDOPHYTIC OVIPOSITION TRACES OF ODONATA (EOCENE, ARGENTINA)" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referees’ reports. Please find the referees' comments along with any feedback from the Editors below my signature.

We invite you to respond to the comments and revise your manuscript. Below the referees’ and Editors’ comments (where applicable) we provide additional requirements. Final acceptance of your manuscript is dependent on these requirements being met. We provide guidance below to help you prepare your revision.

Please submit your revised manuscript and required files (see below) no later than 7 days from today’s (ie 19-Oct-2020) date. Note: the ScholarOne system will ‘lock’ if submission of the revision is attempted 7 or more days after the deadline. If you do not think you will be able to meet this deadline please contact the editorial office immediately.

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Thank you for submitting your manuscript to Royal Society Open Science and we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best regards,

Lianne Parkhouse
Editorial Coordinator
Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Jeffrey Thompson (Associate Editor) and Kevin Padian (Subject Editor)
openscience@royalsociety.org

Associate Editor Comments to Author (Dr Jeffrey Thompson):

Dear Eugenia et al,

Your manuscript has been reviewed by three expert reviewers, and I am happy to say that they have all been very positive about your manuscript. I am thus recommending acceptance with minor revisions. There are a few cases where things are not clear in the manuscript, where things are misspelled, or where the the English could be improved, which has been highlighted by the reviewers in their attached, annotated, files. There are also a number of stylistic errors found in the references. I thus suggest you go through the manuscript with a fine-toothed-comb to correct these errors in spelling, formatting, and clarity prior to resubmission. Please address all suggestions and comments made by the reviewers prior to resubmission.

All the best,
Jeff Thompson

Reviewer comments to Author:

Reviewer: 1
Comments to the Author(s)

I included some corrections and comments in the pdf file of the manuscript (attached)

Reviewer: 2
Comments to the Author(s)

RSOS-201126

GEOMETRIC MORPHOMETRICS OF ENDOPHYTIC OVIPOSITION TRACES OF ODONATA (EOCENE, ARGENTINA)

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Illustration and the effort of represent each oviposition per leaf in a draw are very good.

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Author's Response to Decision Letter for (RSOS-201126.R0)

See Appendix C.
We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Romero-Lebrón,

It is a pleasure to accept your manuscript entitled "GEOMETRIC MORPHOMETRICS OF ENDOPHYTIC OVIPOSITION TRACES OF ODONATA (EOCENE, ARGENTINA)" in its current form for publication in Royal Society Open Science.

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Thank you for your fine contribution. On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,
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| Journal                  | Royal Society Open Science |
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| Manuscript ID            | RSOS-201126                |
| Article Type             | Research                   |
| Date Submitted by the Author | 21-Aug-2020              |
| Complete List of Authors | Romero-Lebrón, Eugenia; IMBIV GLEISER, RAQUEL M.; IMBIV PETRULEVIČIUS, JULIÁN F.; Facultad de Ciencias Naturales; CONICET |
| Subject                  | palaeontology < BIOLOGY   |
| Keywords                 | ENDOPHYTIC, OVIPOSITION, TRACES, ODONATA, GEOMETRIC MORPHOMETRICS |
| Subject Category         | Organismal and Evolutionary Biology |

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Statement (if applicable):
Our data are deposited at Dryad: https://doi.org/10.5061/dryad.d51c5b015.
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I/We declare we have no competing interests

Statement (if applicable):
CUST.STATE.CONFLICT :No data available.

Authors’ contributions

This paper has multiple authors and our individual contributions were as below

Statement (if applicable):
Authors’ contributions. E.R-L. took the photographs, did the analysis, interpretation and drafted the first version of the manuscript with intellectual input from all other authors. All authors contributed intellectually critical content and revisions. All authors gave final approval for the final version.
GEOMETRIC MORPHOMETRICS OF ENDOPHYTIC OVIPOSITION TRACES OF ODONATA (EOCENE, ARGENTINA)

by EUGENIA ROMERO-LEBRÓN¹*, RAQUEL M. GLEISER¹ and JULIÁN F. PETRULEVIČIUS²

¹ Centro de Relevamiento y Evaluación de Recursos Agrícolas y Naturales (IMBIV, UNC-CONICET). 5000, Córdoba. eugeniaromerolebron@gmail.com; raquel.gleiser@unc.edu.ar
² División Paleozoología Invertebrados, Facultad de Ciencias Naturales y Museo (UNLP), and CONICET. 1900, La Plata, Buenos Aires. levicius@fcnym.unlp.edu.ar

*Corresponding author

OCCASIONALLY, plants serve as substrates for insect oviposition. In fossil leaves endophytic oviposition is revealed by the presence of scars (traces) on the surface, which is generated in response to the lesion caused by the ovipositor (1-5).

Since the Paleozoic there is evidence of endophytic oviposition in fossil leaves (6, 7). The traces are generally oval (5, 6, 8, 9) and are usually arranged on the leaf according to a pattern. Based on the oviposition pattern followed by the traces, these are classified (among others) as *Paleoovoidus rectus* if the traces are arranged along a linear pattern, *Paleoovoidus bifurcatus* if they occur in pairs forming double or V-shaped rows and *Paleoovoidus arcuatus* when they describe a curved or zigzag pattern. The *Paleoovoidus* ichnogenus proposed by Vasilenko (2) is characterized by medium-sized, elongated, narrow, ovoid or lens-shaped structures with a regular arrangement on the leaf blade. These traces, defined by the reaction tissue of the leaf, are narrow at one end, and are often presented as a dark spot (definition proposed by Vasilenko (2) and redefined by Sarzetti (3). At present, more than a dozen species are included in this ichnogenus (10).

Odonatoptera is one of the oldest lineages of winged insects (Pterygota) that reaches today. They are recorded from the Lower Carboniferous (325 Ma) with already 4 orders (11); and have a relatively rich fossil record (12). Odonates are predatory insects, with aquatic or semi-aquatic nymphs. Sexually active Odonata
gather in or around bodies of water to mate and lay their eggs. They have two oviposition strategies: endophytic and exophytic (12). In endophytic behaviour eggs are inserted into plant tissues, while exophytic females release eggs in the water or deposit them on the surface of periaquatic objects (13). After mating, the female with endophytic oviposition selects a plant substrate and lacerates the plant tissue with the cutting shells in its ovipositor to generate a cavity where the egg is inserted.

Insect eggs come in an incredible diversity of shapes and sizes (14-16). Variations in egg size can be found within different populations [e.g. (17, 18)] or even between eggs from the same clutch of a female [e.g. (19)]. Egg size also varies temporally and spatially, and may be related to changes in population quality and abundance (20). Among odonates, species differ in reproductive traits such as egg size, which is an important feature, as it affects larval size and developmental performance (19). Corkum (21) showed that hatchlings from larger eggs are larger than those from smaller eggs. The size of immature stages is relevant if we consider that in Odonata cannibalism and predation are highly represented, with larger individuals feeding on smaller ones (22-25).The shape of individual oviposition traces has not been evaluated except by Romero-Lebrón (26) who showed that the traces have a range of shapes that are consistent with position differences of the female due to oviposition behaviour. This study was conducted on a leaf of Eucalyptus chubutensis (Berry) González (in part), (27) (Myrtaceae) from Laguna del Hunco (Chubut, Argentina) (Early Eocene) that showed traces of individual Odonata eggs that were previously classified to two ichnospecie [P. arcuatus and P. rectus, (3)], which would have been performed by a single female.

Despite the efforts of Romero-Lebrón (26), it is still not clear whether the shape of individual traces varies in relation to factors such as ichnological classification, age of the fossil or taxonomic identification of the substrate. The aim of this study is to carry out a detailed study of the shape and size of individual endophytic oviposition traces of Lower and Middle Eocene fossil odonates in Patagonia, Argentina, and thus to elucidate whether there are changes in shape or size of the individual traces between these two periods, and/or if they are associated with the substrate used at
the time of oviposition, and the ichnological classification. This is an unprecedented large-scale study and for this purpose we have considered the geometric morphometric (by Fourier elliptical analysis) and the classical morphometrics of the individual oviposition traces.

MATERIALS AND METHODS

The complete collection of oviposition traces of the Egidio Feruglio Paleontological Museum (MEF), located in Trelew, Province of Chubut, Argentina, was reviewed. We photographed and studied in detail 24 materials that possessed traces of endophytic ovipositions attributed to Odonata, 23 of which are published in Sarzetti (3). The materials have two collection numbers, one paleobotanical (MPEF-Pb) and one ichnological (MPEF-IC). MPEF-Pb-2216 has two ichnological classifications (MPEF-IC-1376 and MPEF-IC-1392). The traces come from the Patagonian Eocene localities of Laguna del Hunco [Ypresian, 52 Ma; (28)] and Río Pichileufú [Lutetian, 48 Ma; (28)] (Fig. 1), both well-known localities concerning their Odonata (29-37), and plant diversity (28, 38-40).

The materials were observed and photographed with a Nikon SMZ1000 magnifier with built-in Nikon DS-Fi1-L2 camera. For this purpose, the leaf fossils containing traces of endophytic ovipositions were placed on a horizontal support, perpendicular to the camera’s CCD. The distance between the objective of the camera and the sample to be photographed was held constant to avoid distortion of the micro-photographs, which were generated in a 2560 x 1920 pixels size in TIF format. The images were edited so that all the partial photographs completed the entire leaf. The contours of each trace were marked in detail on a digitally superimposed layer (Fig. 1 B). This layer was used in the analysis of classical morphometrics and geometric morphometrics.

Classical morphometric is based on measurements of linear distances, such as length and width. Geometric morphometrics, on the other hand, capture the complete geometry of the organism (41). Although there are numerous mathematical
techniques for describing closed contours [for a review of these, see (42-44)], variants of Fourier analysis are generally used. In general, this technique consists of analysing the contribution of the coefficients of a trigonometric function that reproduces a certain curve as accurately as possible. Fourier analysis coefficients then become descriptors of the shape of that curve that can be compared with the corresponding coefficients of other curves using various multivariate statistical techniques.

Classical morphometrics

The following measurements were taken for each oviposition trace using ImageJ 1.51n: length (measured as the longest area of the trace), width (measured as the widest area of the trace perpendicular to length), area and perimeter (Table 1). The results were analysed using Generalized and Mixed Linear Models (MLGM):

\[ Y = \mu + \tau_{\text{locality/age}} + \tau_{\text{ichnotaxonomy}} + \epsilon_{\text{substrate}} > \epsilon_{\text{material}} + \epsilon \]

where \( Y \) represents the measures of the traces (length, width, etc.). The model has two fixed factors, the "age/locality" factor (with two levels, Laguna del Hunco / Lower Eocene, Río Pichileufú / Middle Eocene) and the "ichnotaxonomy" factor (with three levels, one per ichnotaxon), and two random effects: taxonomic identity of the oviposition substrate (with 10 levels, depending on the lowest level of taxonomic resolution achieved) and identity of the material (25 levels); material was nested in substrate.

On the other hand, the effect of the substrate on the dimensions of the traces was evaluated according to the following model:

\[ Y = \mu + \tau_{\text{substrate}} + \epsilon_{\text{ichnotaxonomy}} + \epsilon_{\text{locality}} + \epsilon_{\text{material}} + \epsilon \]
where substrate was considered a fixed factor and three random factors were included to ichnotaxonomy, locality and material identity.

Considering that the previous model included pseudo replicas for several substrates of which there was only one representative, on the other hand, at family level only Myrtaceae and Proteaceae were compared, since they were the only ones for which there were repetitions, according to the following model:

\[
Y = \mu + \tau_{\text{family}} + \epsilon_{\text{material}} + \varepsilon
\]

where family was considered a fixed factor with two levels (Myrtaceae and Proteaceae) and material as random factor (all this material comes from Laguna del Hunco and were \textit{P. arcuatus}).

In all the models the fulfillment of the assumptions of normality and homocedasticity was verified, and in its defect the correction was carried out by means of the function \texttt{varldent}. In case of significant effects, in order to determine which means differed, the LSD Fisher \textit{a posteriori} test was carried out (Alfa = 0.05).

**Geometric morphometrics**

For geometric morphometric Elliptic analysis of Fourier was performed, following the methodology described in Romero-Lebrón (26). We worked with individual images of the contour of each of the traces (Fig. 1 C). The free statistical package SHAPE 1.3 (45) was used to calculate Fourier coefficients. Twenty harmonics were taken (46) (tested with a lower number of harmonics; 20 harmonics were better adjusted to the reference shape). With the numerous variables produced, a principal component analysis (PCA) was performed using covariance matrices. For
this, the free distribution statistical package PAST 3.15 was used (47). Finally, PrinPrint (SHAPE 1.3 subprogram) was used to visualize the variation in shape represented by each principal component.

RESULTS

A total of 1346 traces of Odonata oviposition from 24 materials were studied (traces that were not well defined and had incomplete contours were excluded). As MPEF-Pb-2216 has two ichnospecies in the same leaf (MPEF-IC-1376 and MPEF-IC-1392) and the analyses were carried out based on ichnological classifications, the final number of samples is 25.

The sample MEF-IC-1382, which was originally unidentified, was classified by us as *P. arcuatus* according to Krassilov (48) because the traces follow a curved pattern (Appendix S1). The substrate was assigned to a dicotyledonous leaf.

Based on the botanical taxonomy of the material (information provided by the MEF except for the sample MEF-IC-1382), a great diversity of substrates used for oviposition is observed. Traces of oviposition were found in 12 Dicotyledons without minor identification, and in 7 families: Malvaceae, Myrtaceae, Celtidaceae, Cunoniaceae, Fabaceae, Proteaceae, and Sapindaceous (Table 1).

Regarding the abundance of substrates used for endophytic oviposition, 50 % of the material was only identified at the Dicotyledon Class level; for the Celtidaceae (*Celtis ameghenoi* Berry), Cunoniaceae (*Cupania latifolioides* Berry), Fabaceae (*Cassia argentinensis* Berry), Malvaceae and Sapindaceous families (*Schmidelia proedulis* Berry) 1 material of each was found. The Myrtaceae family was the most represented (*Myrica deltodea* Berry, *Eucalyptus chubutensis* Berry) followed by Proteaceae (*Lomatia occidentalis* Berry) (Table 1).

As for the ichnotaxonomic classification already attributed to the reviewed materials, there were three ichnospecies: *Paleoovoidus arcuatus*, *Paleoovoidus bifurcatus* and *Paleoovoidus rectus* (Table 1). The ichnospecies *P. arcuatus* was the
most frequent (n = 22) representing 91.67 % of total materials, followed by P. bifurcatus and P. rectus, each with only 1 specimen, and one unidentified material now assigned to P. arcuatus. Paleoovoidus arcuatus is present in a great diversity of substrates. On one occasion, P. arcuatus coexists with P. rectus in a single specimen of the family Myrtaceae (Eucalyptus chubutensis) [Romero-Lebrón (26) analyse this material in detail]. The only specimen of P. bifurcatus is found in the only sample with traces of the family Sapindaceous (Schmidelia proedulis) (Table 1).

Classical Morphometrics

Relationship of metrics with provenance (age), ichnotaxonomic classification and oviposition substrate. The traces from Río Pichileufú (Middle Eocene) were significantly wider and of larger area than those from Laguna del Hunco (Lower Eocene) (p < 0.05). The traces of greater length, area and perimeter are those of P. bifurcatus (MPEF-IC-1385: 1.75 ± 0.13 mm), while those of P. arcuatus and P. rectus do not differ from each other (p < 0.05). Material MPEF-IC-1382 (which we assigned to P. arcuatus) has a trace length similar to P. arcuatus and P. rectus (Fig. 2). The substrate with traces of greater (p < 0.05) length, area and perimeter is Schmidelia proedulis (classified as P. bifurcatus); while the other substrates in general show relatively little variation in all dimensions (Fig. 3). There were also no significant differences in trace dimensions between the families Myrtaceae and Proteaceae.

Geometric Morphometrics

For each of the 25 samples a principal component analysis (PCA) of their Fourier coefficients was performed to describe the shape of the individual traces (Fig. 4. Appendix S2-S5). The principal component 1 explained between 43.33 % and 98.81 % of the total variation of the data (Table 2). In 84 % of the material the shape close to the apex was discriminated, while in 12 % the apex shape was discriminated.
principal component 2 explained between 0.73 % and 36.32 % of the total variation of the data, discriminating the curvature of the trace.

Ichnotaxonomy and geometric morphometrics

The PCA of Fourier coefficients in relation to trace ichnotaxonomy reduced their variability to three principal components (PC) which together explain 92.61 % of the total variation observed in the shape. PC 1, PC 2 and PC 3 respectively explain 58.06 %, 26.83 % and 7.72 % of the total variation. A great diversity of oviposition trace morphotypes is observed in *P. arcuatus*. However, no particular trace shape was detected for each taxon, as the morphotypes observed in *P. arcuatus* are shared with *P. rectus* and *P. bifurcatus* (Fig. 5).

Substrate diversity and geometric morphometrics

The first three principal components of the PCA of Fourier coefficients in relation to substrate diversity together explain 92.66 % of the total variance in the shape. PC 1, 2 and 3 explain 64.54 %, 21.08 % and 7.04 % of the total variation, respectively. We assessed the 12 materials identified at family or species level. Those assigned only to the category "Dicotyledonous" were excluded in this instance of analysis (see Table 1 for details of the materials). No particular morphotype is observed for each substrate (Fig. 6).

Comparison between Lower and Middle Eocene

The analysis of principal components on the Fourier coefficients considering the ages/localities of the samples reduced the variability to three principal components (PC) that together capture 92.61 % of the total variance observed in the shape. PC 1, 2 and 3 explain 58.06 %, 26.83 % and 7.72 % of the total variation, respectively. A large overlap of morphotypes is observed, regardless of age/locality (Fig. 7).
DISCUSSION

Substrate

The choice of substrate and environment that adults choose as a place for the development of their offspring is complex and important, as it will influence the reproductive success of the species (49). Vegetation composition is considered to be an important indicator for current odonates for habitat selection of future larvae (50, 51). In fossil material, there are some authors assigning substrate preferences for oviposition to certain families of Zygoptera. According to Hellmund & Hellmund (52, 53) Lestidae females often show substrate preference for certain plants such as *Daphnogene* leaves (Lauraceae), while Coenagrionidae females, however, do not appear to prefer a plant type in the fossil record. In this respect, we have to be careful to make attributions to Recent families for ovipositions in the Eocene as there are extinct families of Odonata (29), like Frenguelliidae, Austroperilestidae and Burmagomphidae in Laguna del Hunco and Río Pichileufú (30, 31, 33, 36) that could be also the producers (29). Concerning a more reliable attribution of ovipositions to a certain genus or family of plants, in our studies, it is difficult to assign a preference for substrate because most plant taxa were represented by one or a few samples. However, all substrates with traces of oviposition analysed correspond to Dicotyledonea. The most represented family is Myrtaceae, being *Eucalyptus chubutensis* (Myrtaceae) and *Lomatia occidentalis* (Proteaceae) the most abundant species. Wilf (38, 39) analysed in detail the diversity of the flora of Laguna del Hunco and Río Pichileufú based on more than 3500 fossil materials, providing a list of the thirty most abundant plant species of Laguna del Hunco. The substrates in which traces were detected belong to taxa of plants included in this list, therefore, the abundance of substrates chosen for oviposition, agrees with the relative abundance in the environment, then there would be no evidence of a pressure of selection of substrate. On the other hand, *Myrcia deltodea* is not one of the 30 most frequent plants, opening the question as to whether it reflects a selection by this substrate.
Classical Morphometrics

Historically, linear measurements have been widely used to describe fossil structures. Sarzetti (3) provide measurements of some of the materials analysed in this study that differ from our estimates. The differences between Sarzetti (3) and our measurements could be due in part to the fact that they considered different numbers of traces, for example, in the material identified as \textit{P. rectus} (MPEF-IC-1376) they considered twelve traces, while we identified thirteen. However, four of them were excluded from the analyses because they were incomplete. In the case of \textit{P. arcaucus}, the differences could be due to the fact that they do not consider the material MPEF-IC-1382, but instead material (USNM 40389) belonging to the National Museum of Natural History, Smithsonian Institution (Washington, DC) not evaluated by us. In the case of \textit{P. bifurcatus}, the differences observed could be due to different criteria when measuring the trace. In fact, differences of criteria in the measurement are a frequent problem for those who work with linear measurements that could be reduced using the technique of geometric morphometric.

Ichnotaxonomy

\textit{Paleoovoidus arcaucus} is the ichnotaxa most abundant in Laguna del Hunco and Rio Pichileufú. The ichnogenus \textit{Paleoovoidus} Vasilenko (2) was originally described for traces of oviposition belonging to the Upper Jurassic - Lower Cretaceous of Russia. Since then it is presented in great abundance, mainly in the Paleogene and Neogene (manuscript in preparation). Authors such as Sarzetti (3) and Petrulevičius (4, 29), cite \textit{P. arcaucus} in the Eocene and Oligocene, but there could be numerous synonyms if we consider only the spatial arrangement of the set of traces (e.g. "Coenagrionidae Type" or DT54 and DT 100) that would identify it from the Permian [e.g. (54)] and even from the Carboniferous [e.g. (6)]. On one occasion \textit{P. arcaucus} and \textit{P. rectus} were presented in the same leaf [MPEF-Pb-2216 (MPEF-IC-1376 and MPEF-IC-1392)], being interpreted as traces made by one female (26).
Of the three ichnotaxa present, traces of *P. bifurcatus* were the longest. This ichnospecie is mentioned for the first time in Sarzetti (3). Oviposition traces of insects with a pattern similar to *P. bifurcatus* are found in the Lower Permian of India (55), but the size of the traces is considerably smaller (0.1 to 0.5 mm). Later, Hellmund & Hellmund (52, 53, 56) and Petrulevičius (4) report traces of Odonata oviposition in Upper Oligocene materials from Germany with this type of spatial arrangement, also in smaller size (0.5 to 0.9 mm). Considering only the spatial arrangement of the traces of insect oviposition, in the "Curved", "Straight" and "Bifurcated" arrangement, the "Bifurcated" is recorded from Permian to Oligocene (except Paleocene) (manuscript in preparation). On the other hand, traces of oviposition of insects with a similar length range are recorded in the Permian (57-60), Triassic (10, 61), Jurassic (62), Cretaceous (63), Paleocene (64), Oligocene (53, 65, 66) and Miocene (56), but in none of these cases do the traces have spatial arrangement similar to *P. bifurcatus*. Therefore, the characteristics of this ichnospecie could be unique to Argentinian Patagonia, although we would need more samples to reinforce this hypothesis.

The number of oviposition traces per leaf in the studied materials is very variable, with a minimum - maximum range of 5 to 294 traces. In the bibliography, from Cretaceous onwards the number of endophytic ovipositions per leaf increases considerably (manuscript in preparation). Krassilov (67) cite for the Lower Cretaceous of Israel, 250 traces of oviposition of Zygoptera in a Dicotyledonous leaf, and from that moment on, it is not strange to find high amounts of ovipositions, like those found in our study.

**Geometric morphometric**

While numerous papers have contributed trace data, most descriptions of traces of fossil eggs have been qualitative, based on linear dimensions (length-width). For the first time, a large number of Odonata’s endophytic egg traces are analysed with geometric morphometric, except for the Romero-Lebrón (26) observation, but their analysis was done on a single leaf. Using geometric morphometric we observed a
great diversity of oviposition trace morphotypes in the ichnospecie *P. arcuatus*. These morphotypes are shared with *P. rectus* and *P. bifurcatus*. The diversity of morphotypes of *P. arcuatus* could be due to the great abundance of identified materials of this ichnotaxon, since of *P. rectus* and *P. bifurcatus* there is only one representative of each. The greatest variation in the shape of the oviposition traces corresponded to the shape close to the apex, and to a lesser extent to the curvature and convexity of the trace. These three variations are also observed in the morphology of current Zygoptera eggs (manuscript in preparation).

On the other hand, no particular morphotype was observed for each oviposition substrate. Regardless of the substrate, the average shape of the traces does not vary. Therefore, we interpreted that the substrate would not be exerting shape modification pressures on the oviposition traces of the studied materials. In addition, a marked overlap was observed in the shape of the traces regardless of their locality of origin (LH - Lower Eocene - and RP - Middle Eocene -), so it could be inferred that the shape of the individual traces would not depend on provenance or age. Therefore, the variations in the shape of the individual traces would not be related to the ichnotaxonomy, substrate used or locality of provenance or age.

In summary, the shapes of Odonata’s individual egg traces are observed to be stable over 4 million years (Lower Eocene-Middle Eocene), while individual size varies. Insect eggs exhibit a great diversity of sizes, these variations are observed within populations (17, 18) and there are even variations in the eggs that the same female oviposes [e.g. (19)]. Therefore egg size results in a highly variable parameter that is also observed in the traces of Patagonia Odonata (Lower-Middle Eocene). On the other hand, in this work no consistent changes in egg shape are observed despite the different substrates used for oviposition, the 4 million years considered in this study and this is not reflected by the ichnotaxonomy either. According to Legay (15) insects have existed for hundreds of millions of years, but despite their great diversification, the shape of their eggs shows great stability. As far as we know, this is the first work that analyzes the change of shape of Odonata's endophytic eggs in a scale of 4 million years and therefore, it would be the first work that empirically
reflects the stability of shape proposed by Legay (15). This could reflect that the
shape of Odonata eggs, unlike their size, could have a strong evolutionary constrain
already observed since the Patagonian Eocene.

Data accessibility. Our data are deposited at Dryad: https://doi.org/10.5061/dryad.d51c5b015. Dryad review URL https://datadryad.org/stash/share/VFMiABxzw5HFORolcfipntAxkOMQtX0dUuC5319sJJ4.

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Supporting information labels:

Appendix S1. Material MPEF-IC-1382. A) Original material; B) Partial photographs taken with the magnifying glass completing the whole sheet. It has an overlapping layer in which the contours of each trace were marked and identified with a number; C) Detail of the layer with marked and identified traces; D) Principal component analysis graph PC 1 (59.41 %) - PC 2 (32.41 %) with 95% confidence ellipse. Scales: A) and B) 1 cm, C) 1x1 mm black square.

Appendix S2. Material MEF-IC-1376. A) Original material; B) Partial photographs taken with a magnifying glass completing the whole leaf. It has an overlapping layer in which the contours of each trace were marked and identified with a number; C) Detail of the layer with marked...
and identified traces; D) Principal component analysis graph PC 1 (60.06 %) - PC 2 (30.30 %) with 95% confidence ellipse. Scales: A) and B) 1 cm, C) 1x1 mm black square.

Appendix S3. Material MEF-IC-1385. A) Original material; B) Partial photographs taken with a magnifying glass completing the whole leaf. It has an overlapping layer in which the contours of each trace were marked, which were identified with a number; C) Detail of the layer with marked and identified traces; D) Principal component analysis graph PC 1 (87.44 %) - PC 2 (7.55 %) with 95% confidence ellipse. Scales: A) and B) 1 cm, C) 1x1 mm black square.

Appendix S4. Material MEFP-IC-1388. A) Original material; B) Partial photographs taken with a magnifying glass completing the whole leaf. It has an overlapping layer in which the contours of each trace were marked, which were identified with a number; C) Detail of the layer with marked and identified traces; D) Principal component analysis graph PC 1 (57.06 %) - PC 2 (23.80 %) with 95% confidence ellipse. Outliers traces numbers: 31, 33, 40, 95, 98 and 240. Scales: 1) and 2) 1 cm, 3) 1x1 mm black square.

Appendix S5. Material MEF-IC-1378. A) Original material; B) Partial photographs taken with a magnifying glass completing the whole leaf. It has an overlapping layer in which the contours of each trace were marked, which were identified with a number; C) Detail of the layer with marked and identified traces; D) Principal component analysis graph PC 1 (55.01 %) - CP 2 (27.10 %) with 95% confidence ellipse. Outliers trace numbers: 45, 48, 50, 54, 71 and 72. Scales: A) and B) 1 cm, C) 1x1 mm black square.
Figure 1. A) Image of the Dicotyledonous leaf with traces of Odonata endophytic eggs located along the entire leaf surface (MPEF-IC-1388), scale: 1 cm. B) Superposition of layers to the main photograph of the leaf in which the contours of each trace were marked in detail. C) Detail of an egg trace with the scale (square 1 x 1 mm) in the lower margin. D) Map of Argentina showing the localities of Río Pichileufú and Laguna del Hunco in the Patagonia.

165x160mm (300 x 300 DPI)
Figure 2. Classical morphometrics of the traces of Odonata endophytic oviposition in relation with their ichnotaxonomy (A-E) and for *Paleoooidus arcuatus*, in relation with the age/locality (F-J). Mean values ± standard errors are displayed. Different letters between columns indicate statistically significant differences (p < 0.05).

165x200mm (300 x 300 DPI)
Figure 3. Classical morphometrics of the oviposition traces of Odonata in relation with the substrate used. Average + standard error shown. Different letters between columns indicate statistically significant differences (p < 0.05).

165x282mm (300 x 300 DPI)
Figure 4. Variation of shape in the traces of oviposition in fossil leaves from Laguna del Hunco and Río Pichileufú according to ichnotaxon. MPEF-IC 1376 Paleoovoidus rectus (Laguna del Hunco); MPEF-IC 1386 Paleoovoidus bifurcatus (Laguna del Hunco); MPEF-IC 1388 Paleoovoidus arcuatus (Río Pichileufú); MPEF-IC 1378 Paleoovoidus arcuatus (Laguna del Hunco). The variation in shape is represented in standard deviation units (-2 and +2) and the mean shape is the mean of the Fourier coefficients for all forms analysed.

*165x94mm (300 x 300 DPI)*
Figure 5. Analysis of the principal components of Fourier coefficients, of the individual traces analysed in 25 samples in relation to the 3 identified ichnotaxa (black dots correspond to MPEF-IC-1382 material assigned to *P. arcuatus*).

165x88mm (300 x 300 DPI)
Figure 6. Analysis of the principal components of Fourier coefficients, of the individual traces analysed in relation to the 12 oviposition substrates identified at family or species level.

165x88mm (300 x 300 DPI)
Figure 7. Analysis of the principal components of the Fourier coefficients, of the individual traces analysed in 25 samples in relation to their origin: Laguna del Hunco (Lower Eocene) and Río Pichileufú (Middle Eocene).

165x88mm (300 x 300 DPI)
Table 1. Materials analysed: identification, locality (RP = Río Pichileufú; LH = Laguna del Hunco), paleobotanical classification, number of traces and measurements (Average ± Standard Error). Values are expressed in mm. P. = *Paleoovoidus*.

| MPEF-IC | Location | Substrate     | Ichnospecies |
|---------|----------|---------------|--------------|
| 1386    | RP       | Dicotyledonous | *P. arcuatus*|
| 1388    | RP       | Dicotyledonous | *P. arcuatus*|
| 1390    | RP       | Dicotyledonous | *P. arcuatus*|
| 1391    | RP       | Dicotyledonous | *P. arcuatus*|
| 1393    | RP       | Dicotyledonous | *P. arcuatus*|
| 1382    | LH       | Dicotyledonous | *P. arcuatus*|
| 1367    | LH       | Dicotyledonous | *P. arcuatus*|
| 1371    | LH       | Dicotyledonous | *P. arcuatus*|
| 1372    | LH       | Dicotyledonous | *P. arcuatus*|
| 1375    | LH       | Dicotyledonous | *P. arcuatus*|
| 1380    | LH       | Dicotyledonous | *P. arcuatus*|
| 1383    | LH       | Dicotyledonous | *P. arcuatus*|
| 1384    | LH       | Dicotyledonous | *P. arcuatus*|
| 1381    | LH       | Malvaceae     | *P. arcuatus*|
| 1370    | LH       | Celtidaceae   | *Celtis ameghenoi* | *P. arcuatus*|
| 1374    | LH       | Cunoniaceae   | *Cupania latifolioides* | *P. arcuatus*|
| 1377    | LH       | Fabaceae      | *Cassia argentinensis* | *P. arcuatus*|
| 1378    | LH       | Proteaceae    | *Lomatia occidentalis* | *P. arcuatus*|
| 1389    | LH       | Sapindaceous  | *Schmidelia proedulis* | *P. bifurcatus*|
| 1385    | LH       | Myrtaceae     | *Myrcia deltodea* | *P. arcuatus*|
| 1368    | LH       | Eucalyptus chubutensis | *P. arcuatus*|
| 1369    | LH       | Eucalyptus chubutensis | *P. arcuatus*|
| 1373    | LH       | Myrtaceae     | *Eucalyptus chubutensis* | *P. arcuatus*|
| 1376    | LH       | Eucalyptus chubutensis | *P. rectus*|
| 1392    | LH       | Eucalyptus chubutensis | *P. arcuatus*|
Table 1. Materials analysed: identification, locality (RP = Río Pichileufú; LH = Laguna del Hunco), paleobotanical classification, ichnotaxonomic classification, number of traces and measurements (Average ± Standard Error). Values are expressed in mm. P. = Paleoovoidus

| n traces | Length (L)     | Width (W)     | L/W Index | Area (mm²) | Perimeter |
|----------|----------------|---------------|-----------|------------|-----------|
| 8        | 1.28 ± 0.04    | 0.77 ± 0.02   | 1.67 ± 0.05 | 0.75 ± 0.03 | 3.47 ± 0.08 |
| 240      | 0.87 ± 0.01    | 0.33 ± 0.01   | 2.75 ± 0.03 | 0.21 ± 0.01 | 2.02 ± 0.04 |
| 126      | 1.21 ± 0.01    | 0.46 ± 0.01   | 2.65 ± 0.03 | 0.48 ± 0.01 | 3.09 ± 0.03 |
| 58       | 0.81 ± 0.02    | 0.29 ± 0.01   | 2.89 ± 0.07 | 0.17 ± 0.01 | 1.90 ± 0.05 |
| 44       | 1.34 ± 0.02    | 0.55 ± 0.01   | 2.45 ± 0.05 | 0.54 ± 0.02 | 3.32 ± 0.05 |
| 18       | 0.99 ± 0.04    | 0.41 ± 0.02   | 2.44 ± 0.04 | 0.32 ± 0.03 | 2.45 ± 0.11 |
| 294      | 0.95 ± 0.01    | 0.35 ± 0.35   | 2.82 ± 0.03 | 0.26 ± 0.01 | 2.31 ± 0.03 |
| 30       | 0.91 ± 0.02    | 0.31 ± 0.01   | 2.95 ± 0.08 | 0.22 ± 0.01 | 2.19 ± 0.04 |
| 21       | 1.18 ± 0.02    | 0.52 ± 0.01   | 2.29 ± 0.05 | 0.50 ± 0.02 | 3.05 ± 0.05 |
| 36       | 1.12 ± 0.03    | 0.38 ± 0.02   | 3.00 ± 0.10 | 0.33 ± 0.02 | 2.68 ± 0.08 |
| 31       | 0.91 ± 0.03    | 0.33 ± 0.01   | 2.84 ± 0.09 | 0.19 ± 0.01 | 2.09 ± 0.08 |
| 39       | 1.16 ± 0.04    | 0.40 ± 0.02   | 2.97 ± 0.09 | 0.37 ± 0.03 | 2.78 ± 0.10 |
| 21       | 1.07 ± 0.05    | 0.47 ± 0.03   | 2.36 ± 0.09 | 0.39 ± 0.04 | 2.66 ± 0.14 |
| 5        | 0.79 ± 0.10    | 0.25 ± 0.03   | 3.12 ± 0.14 | 0.15 ± 0.03 | 1.83 ± 0.23 |
| 65       | 1.35 ± 0.02    | 0.47 ± 3E⁻³   | 2.85 ± 0.04 | 0.49 ± 0.01 | 3.26 ± 0.03 |
| 38       | 1.00 ± 0.02    | 0.34 ± 0.01   | 3.06 ± 0.10 | 0.26 ± 0.01 | 2.36 ± 0.04 |
| 30       | 0.98 ± 0.03    | 0.35 ± 0.01   | 2.79 ± 0.08 | 0.25 ± 0.01 | 2.33 ± 0.06 |
| 84       | 1.10 ± 0.01    | 0.42 ± 4E⁻³   | 2.63 ± 0.03 | 0.36 ± 0.01 | 2.71 ± 0.03 |
| 49       | 0.77 ± 0.02    | 0.32 ± 0.01   | 2.44 ± 0.06 | 0.17 ± 0.01 | 1.84 ± 0.05 |
| 6        | 1.75 ± 0.13    | 0.62 ± 0.03   | 2.86 ± 0.28 | 0.86 ± 0.08 | 4.27 ± 0.26 |
| 46       | 0.84 ± 0.02    | 0.41 ± 0.01   | 2.11 ± 0.06 | 0.24 ± 0.01 | 2.07 ± 0.06 |
| 4        | 0.79 ± 0.08    | 0.33 ± 0.02   | 2.49 ± 0.37 | 0.19 ± 0.02 | 1.94 ± 0.16 |
| 28       | 0.84 ± 0.04    | 0.35 ± 0.02   | 2.51 ± 0.10 | 0.22 ± 0.02 | 2.05 ± 0.10 |
| 9        | 0.80 ± 0.05    | 0.31 ± 0.02   | 2.63 ± 0.19 | 0.15 ± 0.02 | 1.75 ± 0.11 |
| 16       | 0.84 ± 0.05    | 0.23 ± 0.01   | 3.75 ± 0.20 | 0.12 ± 0.01 | 1.80 ± 0.10 |
and measurements (Average ± Standard Error). Values are expressed in mm. P. = P
Pseudoaleoovoidus
Table 2. Summary table of values of the Principal Components (PC) of the Fourier coefficients.

|       | n  | Mean | S.E. | Min  | Max  | Median |
|-------|----|------|------|------|------|--------|
| **PC 1** | 25 | 62.87| 2.39 | 43.33| 98.81| 60.06  |
| **PC 2** | 25 | 22.71| 1.62 | 0.73 | 36.32| 23.8   |
| **PC 3** | 25 | 7.3  | 0.69 | 0.46 | 14.86| 6.89   |
### Appendix B

#### ROYAL SOCIETY OPEN SCIENCE

**GEOMETRIC MORPHOMETRICS OF ENDOPHYTIC OVIPOSITION TRACES OF ODONATA (EOCENE, ARGENTINA)**

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Does your article include research that required ethical approval or permits?:
This article does not present research with ethical considerations

Statement (if applicable):
CUST_IF_YES_ETHICS :No data available.

Data

It is a condition of publication that data, code and materials supporting your paper are made publicly available. Does your paper present new data?:
Yes

Statement (if applicable):
Our data are deposited at Dryad: https://doi.org/10.5061/dryad.d51c5b015.
Dryad review URL
https://datadryad.org/stash/share/VFMIABxwz5HFORolcfipntAxkOMQtX0dUuC5319sJJ4.

Conflict of interest

I/We declare we have no competing interests

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Authors’ contributions

This paper has multiple authors and our individual contributions were as below

Statement (if applicable):
Authors’ contributions. E.R-L. took the photographs, did the analysis, interpretation and drafted the first version of the manuscript with intellectual input from all other authors. All authors contributed intellectually critical content and revisions. All authors gave final approval for the final version.
GEOMETRIC MORPHOMETRICS OF ENDOPHYTIC OVIPOSITION TRACES OF ODONATA (EOCENE, ARGENTINA)

by EUGENIA ROMERO-LEBRÓN¹*, RAQUEL M. GLEISER¹ and JULIÁN F. PETRULEVIČIUS²

¹ Centro de Relevamiento y Evaluación de Recursos Agrícolas y Naturales (IMBIV, UNC-CONICET). 5000, Córdoba. eugeniaromerolebron@gmail.com; raquel.gleiser@unc.edu.ar
² División Paleozoológía Invertebrados, Facultad de Ciencias Naturales y Museo (UNLP), and CONICET. 1900, La Plata, Buenos Aires. levicius@fcnym.unlp.edu.ar

*Corresponding author

OCCASIONALLY, plants serve as substrates for insect oviposition. In fossil leaves endophytic oviposition is revealed by the presence of scars (traces) on the surface, which is generated in response to the lesion caused by the ovipositor (1-5).

Since the Paleozoic there is evidence of endophytic oviposition in fossil leaves (6, 7). The traces are generally oval (5, 6, 8, 9) and are usually arranged on the leaf according to a pattern. Based on the oviposition pattern followed by the traces, these are classified (among others) as *Paleoovoidus rectus* if the traces are arranged along a linear pattern, *Paleoovoidus bifurcatus* if they occur in pairs forming double or V-shaped rows and *Paleoovoidus arcuatus* when they describe a curved or zigzag pattern. The *Paleoovoidus* ichnogenus proposed by Vasilenko (2) is characterized by medium-sized, elongated, narrow, ovoid or lens-shaped structures with a regular arrangement on the leaf blade. These traces, defined by the reaction tissue of the leaf, are narrow at one end, and are often presented as a dark spot (definition proposed by Vasilenko (2) and redefined by Sarzetti (3). At present, more than a dozen species are included in this ichnogenus (10).

Odonatoptera is one of the oldest lineages of winged insects (Pterygota) that reaches today. They are recorded from the Lower Carboniferous (325 Ma) with already 4 orders (11); and have a relatively rich fossil record (12). Odonates are predatory insects, with aquatic or semi-aquatic nymphs. Sexually active Odonata
gather in or around bodies of water to mate and lay their eggs. They have two oviposition strategies: endophytic and exophytic (12). In endophytic behaviour eggs are inserted into plant tissues, while exophytic females release eggs in the water or deposit them on the surface of periaquatic objects (13). After mating, the female with endophytic oviposition selects a plant substrate and lacerates the plant tissue with the cutting shells in its ovipositor to generate a cavity where the egg is inserted.

Insect eggs come in an incredible diversity of shapes and sizes (14-16). Variations in egg size can be found within different populations [e.g. (17, 18)] or even between eggs from the same clutch of a female [e.g. (19)]. Egg size also varies temporally and spatially, and may be related to changes in population quality and abundance (20). Among odonates, species differ in reproductive traits such as egg size, which is an important feature, as it affects larval size and developmental performance (19). Corkum (21) showed that hatchlings from larger eggs are larger than those from smaller eggs. The size of immature stages is relevant if we consider that in Odonata cannibalism and predation are highly represented, with larger individuals feeding on smaller ones (22-25). The shape of individual oviposition traces has not been evaluated except by Romero-Lebrón (26) who showed that the traces have a range of shapes that are consistent with position differences of the female due to oviposition behaviour. This study was conducted on a leaf of *Eucalyptus chubutensis* (Berry) González (in part), (27) (Myrtaceae) from Laguna del Hunco (Chubut, Argentina) (Early Eocene) that showed traces of individual Odonata eggs that were previously classified to two ichnospecie [P. arcuatus and P. rectus, (3)], which would have been performed by a single female.

Despite the efforts of Romero-Lebrón (26), it is still not clear whether the shape of individual traces varies in relation to factors such as ichnological classification, age of the fossil or taxonomic identification of the substrate. The aim of this study is to carry out a detailed study of the shape and size of individual endophytic oviposition traces of Lower and Middle Eocene fossil odonates in Patagonia, Argentina, and thus to elucidate whether there are changes in shape or size of the individual traces between these two periods, and/or if they are associated with the substrate used at
the time of oviposition, and the ichnological classification. This is an unprecedented large-scale study and for this purpose we have considered the geometric morphometric (by Fourier elliptical analysis) and the classical morphometrics of the individual oviposition traces.

MATERIALS AND METHODS

The complete collection of oviposition traces of the Egidio Feruglio Paleontological Museum (MEF), located in Trelew, Province of Chubut, Argentina, was reviewed. We photographed and studied in detail 24 materials that possessed traces of endophytic ovipositions attributed to Odonata, 23 of which are published in Sarzetti (3). The materials have two collection numbers, one paleobotanical (MPEF-Pb) and one ichnological (MPEF-IC). MPEF-Pb-2216 has two ichnological classifications (MPEF-IC-1376 and MPEF-IC-1392). The traces come from the Patagonian Eocene localities of Laguna del Hunco [Ypresian, 52 Ma; (28)] and Río Pichileufú [Lutetian, 48 Ma; (28)] (Fig. 1), both well-known localities concerning their Odonata (29-37), and plant diversity (28, 38-40).

The materials were observed and photographed with a Nikon SMZ1000 magnifier with built-in Nikon DS-Fi1-L2 camera. For this purpose, the leaf fossils containing traces of endophytic ovipositions were placed on a horizontal support, perpendicular to the camera's CD. The distance between the objective of the camera and the sample to be photographed was held constant to avoid distortion of the micro-photographs, which were generated in a 2560 x 1920 pixels size in TIF format. The images were edited so that all the partial photographs completed the entire leaf. The contours of each trace were marked in detail on a digitally superimposed layer (Fig. 1 B). This layer was used in the analysis of classical morphometrics and geometric morphometrics.

Classical morphometric is based on measurements of linear distances, such as length and width. Geometric morphometrics, on the other hand, capture the complete geometry of the organism (41). Although there are numerous mathematical
techniques for describing closed contours [for a review of these, see (42-44)], variants of Fourier analysis are generally used. In general, this technique consists of analysing the contribution of the coefficients of a trigonometric function that reproduces a certain curve as accurately as possible. Fourier analysis coefficients then become descriptors of the shape of that curve that can be compared with the corresponding coefficients of other curves using various multivariate statistical techniques.

Classical morphometrics

The following measurements were taken for each oviposition trace using ImageJ 1.51n: length (measured as the longest area of the trace), width (measured as the widest area of the trace perpendicular to length), area and perimeter (Table 1). The results were analysed using Generalized and Mixed Linear Models (MLGM):

\[
Y = \mu + \tau_{\text{locality/age}} + \tau_{\text{ichnotaxonomy}} + \rho_{\text{substrate}} > \rho_{\text{material}} + \epsilon
\]

where \( Y \) represents the measures of the traces (length, width, etc.). The model has two fixed factors, the "age/locality" factor (with two levels, Laguna del Hunco / Lower Eocene, Río Pichileufú / Middle Eocene) and the "ichnotaxonomy" factor (with three levels, one per ichnotaxon), and two random effects: taxonomic identity of the oviposition substrate (with 10 levels, depending on the lowest level of taxonomic resolution achieved) and identity of the material (25 levels); material was nested in substrate.

On the other hand, the effect of the substrate on the dimensions of the traces was evaluated according to the following model:

\[
Y = \mu + \tau_{\text{substrate}} + \rho_{\text{ichnotaxonomy}} + \rho_{\text{locality}} + \rho_{\text{material}} + \epsilon
\]
where substrate was considered a fixed factor and three random factors were included to ichnotaxonomy, locality and material identity.

Considering that the previous model included pseudo replicas for several substrates of which there was only one representative, on the other hand, at family level only Myrtaceae and Proteaceae were compared, since they were the only ones for which there were repetitions, according to the following model:

\[
Y = \mu + \tau_{\text{family}} + \sigma_{\text{material}} + \epsilon
\]

where family was considered a fixed factor with two levels (Myrtaceae and Proteaceae) and material as random factor (all this material comes from Laguna del Hunco and were \textit{P. arcuatus}).

In all the models the fulfillment of the assumptions of normality and homocedasticity was verified, and in its defect the correction was carried out by means of the function \texttt{varldent}. In case of significant effects, in order to determine which means differed, the LSD Fisher \textit{a posteriori} test was carried out (Alfa = 0.05).

**Geometric morphometrics**

For geometric morphometric Elliptic analysis of Fourier was performed, following the methodology described in Romero-Lebrón (26). We worked with individual images of the contour of each of the traces (Fig. 1 C). The free statistical package \texttt{SHAPE 1.3} (45) was used to calculate Fourier coefficients. Twenty harmonics were taken (46) (tested with a lower number of harmonics; 20 harmonics were better adjusted to the reference shape). With the numerous variables produced, a principal component analysis (PCA) was performed using covariance matrices.
this, the free distribution statistical package PAST 3.15 was used (47). Finally, PrinPrint (SHAPE 1.3 subprogram) was used to visualize the variation in shape represented by each principal component.

RESULTS

A total of 1346 traces of Odonata oviposition from 24 materials were studied (traces that were not well defined and had incomplete contours were excluded). As MPEF-Pb-2216 has two ichnospecies in the same leaf (MPEF-IC-1376 and MPEF-IC-1392) and the analyses were carried out based on ichnological classifications, the final number of samples is 25.

The sample MEF-IC-1382, which was originally unidentified, was classified by us as *P. arcuatus* according to Krassilov (48) because the traces follow a curved pattern (Appendix S1). The substrate was assigned to a dicotyledonous leaf.

Based on the botanical taxonomy of the material (information provided by the MEF except for the sample MEF-IC-1382), a great diversity of substrates used for oviposition is observed. Traces of oviposition were found in 12 Dicotyledons without minor identification, and in 7 families: Malvaceae, Myrtaceae, Celtidaceae, Cunoniaceae, Fabaceae, Proteaceae, and Sapindaceous (Table 1).

Regarding the abundance of substrates used for endophytic oviposition, 50% of the material was only identified at the Dicotyledonous Class level; for the Celtidaceae (*Celtis ameghenoi* Berry), Cunoniaceae (*Cupania latifolioides* Berry), Fabaceae (*Cassia argentinensis* Berry), Malvaceae and Sapindaceous families (*Schmidelia proedulis* Berry) 1 material of each was found. The Myrtaceae family was the most represented (*Myrcia deltodea* Berry, *Eucalyptus chubutensis* Berry) followed by Proteaceae (*Lomatia occidentalis* Berry) (Table 1).

As for the ichnotaxonomic classification already attributed to the reviewed materials, there were three ichnospecies: *Paleoovoidus arcuatus*, *Paleoovoidus bifurcatus* and *Paleoovoidus rectus* (Table 1). The ichnospecies *P. arcuatus* was the
most frequent (n = 22) representing 91.67 % of total materials, followed by *P. bifurcatus* and *P. rectus*, each with only 1 specimen, and one unidentified material now assigned to *P. arcuatus*. *Paleoovoidus arcuatus* is present in a great diversity of substrates. On one occasion, *P. arcuatus* coexists with *P. rectus* in a single specimen of the family Myrtaceae (*Eucalyptus chubutensis*) [Romero-Lebrón (26) analyse this material in detail]. The only specimen of *P. bifurcatus* is found in the only sample with traces of the family Sapindaceous (*Schmidelia proedulis*) (Table 1).

**Classical Morphometrics**

*Relationship of metrics with provenance (age), ichnotaxonomic classification and oviposition substrate.* The traces from Río Pichileufú (Middle Eocene) were significantly wider and of larger area than those from Laguna del Hunco (Lower Eocene) (p < 0.05). The traces of greater length, area and perimeter are those of *P. bifurcatus* (MPEF-IC-1385: 1.75 ± 0.13 mm), while those of *P. arcuatus* and *P. rectus* do not differ from each other (p < 0.05). Material MPEF-IC-1382 (which we assigned to *P. arcuatus*) has a trace length similar to *P. arcuatus* and *P. rectus* (Fig. 2). The substrate with traces of greater (p < 0.05) length, area and perimeter is *Schmidelia proedulis* (classified as *P. bifurcatus*); while the other substrates in general show relatively little variation in all dimensions (Fig. 3). There were also no significant differences in trace dimensions between the families Myrtaceae and Proteaceae.

**Geometric Morphometrics**

For each of the 25 samples a principal component analysis (PCA) of their Fourier coefficients was performed to describe the shape of the individual traces (Fig. Appendix S2-S5). The principal component 1 explained between 43.33 % and 98.81 % of the total variation of the data (Table 2). In 84 % of the material the shape close to the apex was discriminated, while in 12 % the apex shape was discriminated.
principal component 2 explained between 0.73 % and 36.32 % of the total variation of
the data, discriminating the curvature of the trace.

Ichnotaxonomy and geometric morphometrics

The PCA of Fourier coefficients in relation to trace ichnotaxonomy reduced their
variability to three principal components (PC) which together explain 92.61 % of the
total variation observed in the shape. PC 1, PC 2 and PC 3 respectively explain 58.06
%, 26.83 % and 7.72 % of the total variation. A great diversity of oviposition trace
morphotypes is observed in *P. arcuatus*. However, no particular trace shape was
detected for each taxon, as the morphotypes observed in *P. arcuatus* are shared with
*P. rectus* and *P. bifurcatus* (Fig. 5).

Substrate diversity and geometric morphometrics

The first three principal components of the PCA of Fourier coefficients in relation to
substrate diversity together explain 92.66 % of the total variance in the shape. PC 1,
2 and 3 explain 64.54 %, 21.08 % and 7.04 % of the total variation, respectively. We
assessed the 12 materials identified at family or species level. Those assigned only to
the category "Dicotyledonous" were excluded in this instance of analysis (see Table 1
for details of the materials). No particular morphotype is observed for each substrate
(Fig. 6).

Comparison between Lower and Middle Eocene

The analysis of principal components on the Fourier coefficients considering the
ages/localities of the samples reduced the variability to three principal components
(PC) that together capture 92.61 % of the total variance observed in the shape. PC 1,
2 and 3 explain 58.06 %, 26.83 % and 7.72 % of the total variation, respectively. A
large overlap of morphotypes is observed, regardless of age/locality (Fig. 7).
DISCUSSION

Substrate

The choice of substrate and environment that adults choose as a place for the development of their offspring is complex and important, as it will influence the reproductive success of the species (49). Vegetation composition is considered to be an important indicator for current odonates for habitat selection of future larvae (50, 51). In fossil material, there are some authors assigning substrate preferences for oviposition to certain families of Zygoptera. According to Hellmund & Hellmund (52, 53) Lestidae females often show substrate preference for certain plants such as Daphnogene leaves (Lauraceae), while Coenagrionidae females, however, do not appear to prefer a plant type in the fossil record. In this respect, we have to be careful to make attributions to Recent families for ovipositions in the Eocene as there are extinct families of Odonata (29), like Frenguelliidae, Austroperilestidae and Burmagomphidae in Laguna del Hunco and Río Pichileufú (30, 31, 33, 36) that could be also the producers (29). Concerning a more reliable attribution of ovipositions to a certain genus or family of plants, in our studies, it is difficult to assign a preference for substrate because most plant taxa were represented by one or a few samples. However, all substrates with traces of oviposition analysed correspond to Dicotyledonea. The most represented family is Myrtaceae, being Eucalyptus chubutensis (Myrtaceae) and Lomatia occidentalis (Proteaceae) the most abundant species. Wilf (38, 39) analysed in detail the diversity of the flora of Laguna del Hunco and Río Pichileufú based on more than 3500 fossil materials, providing a list of the thirty most abundant plant species of Laguna del Hunco. The substrates in which traces were detected belong to taxa of plants included in this list, therefore, the abundance of substrates chosen for oviposition, agrees with the relative abundance in the environment, then there would be no evidence of a pressure of selection of substrate. On the other hand, Myrcia deltodea is not one of the 30 most frequent plants, opening the question as to whether it reflects a selection by this substrate.
Historically, linear measurements have been widely used to describe fossil structures. Sarzetti (3) provide measurements of some of the materials analysed in this study that differ from our estimates. The differences between Sarzetti (3) and our measurements could be due in part to the fact that they considered different numbers of traces, for example, in the material identified as *P. rectus* (MPEF-IC-1376) they considered twelve traces, while we identified thirteen. However, four of them were excluded from the analyses because they were incomplete. In the case of *P. arcuatus*, the differences could be due to the fact that they do not consider the material MPEF-IC-1382, but instead material (USNM 40389) belonging to the National Museum of Natural History, Smithsonian Institution (Washington, DC) not evaluated by us. In the case of *P. bifurcatus*, the differences observed could be due to different criteria when measuring the trace. In fact, differences of criteria in the measurement are a frequent problem for those who work with linear measurements that could be reduced using the technique of geometric morphometric.

Ichnotaxonomy

*Paleoovoidus arcuatus* is the ichnotaxa most abundant in Laguna del Hunco and Rio Pichileufú. The ichnogenus *Paleoovoidus* Vasilenko (2) was originally described for traces of oviposition belonging to the Upper Jurassic - Lower Cretaceous of Russia. Since then it is presented in great abundance, mainly in the Paleogene and Neogene (manuscript in preparation). Authors such as Sarzetti (3) and Petrulevičius (4, 29), cite *P. arcuatus* in the Eocene and Oligocene, but there could be numerous synonyms if we consider only the spatial arrangement of the set of traces (e.g. “Coenagrionidae Type” or DT54 and DT 100) that would identify it from the Permian (e.g. (54)) and even from the Carboniferous [e.g. (6)]. On one occasion *P. arcuatus* and *P. rectus* were presented in the same leaf [MPEF-Pb-2216 (MPEF-IC-1376 and MPEF-IC-1392)], being interpreted as traces made by one female (26).
Of the three ichnotaxa present, traces of *P. bifurcatus* were the longest. This ichnospecie is mentioned for the first time in Sarzetti (3). Oviposition traces of insects with a pattern similar to *P. bifurcatus* are found in the Lower Permian of India (55), but the size of the traces is considerably smaller (0.1 to 0.5 mm). Later, Hellmund & Hellmund (52, 53, 56) and Petrulevičius (4) report traces of Odonata oviposition in Upper Oligocene materials from Germany with this type of spatial arrangement, also in smaller size (0.5 to 0.9 mm). Considering only the spatial arrangement of the traces of insect oviposition, in the "Curved", "Straight" and "Bifurcated" arrangement, the "Bifurcated" is recorded from Permian to Oligocene (except Paleocene) (manuscript in preparation). On the other hand, traces of oviposition of insects with a similar length range are recorded in the Permian (57-60), Triassic (10, 61), Jurassic (62), Cretaceous (63), Paleocene (64), Oligocene (53, 65, 66) and Miocene (56), but in none of these cases do the traces have spatial arrangement similar to *P. bifurcatus*. Therefore, the characteristics of this ichnospecie could be unique to Argentinian Patagonia, although we would need more samples to reinforce this hypothesis.

The number of oviposition traces per leaf in the studied materials is very variable, with a minimum - maximum range of 5 to 294 traces. In the bibliography, from Cretaceous onwards the number of endophytic ovipositions per leaf increases considerably (manuscript in preparation). Krassilov (67) cite for the Lower Cretaceous of Israel, 250 traces of oviposition of Zygoptera in a Dicotyledonous leaf, and from that moment on, it is not strange to find high amounts of ovipositions, like those found in our study.

**Geometric morphometric**

While numerous papers have contributed trace data, most descriptions of traces of fossil eggs have been qualitative, based on linear dimensions (length-width). For the first time, a large number of Odonata's endophytic egg traces are analysed with geometric morphometric, except for the Romero-Lebrón (26) observation, but their analysis was done on a single leaf. Using geometric morphometric we observed a
great diversity of oviposition trace morphotypes in the ichnospecie \textit{P. arcuatus}. These morphotypes are shared with \textit{P. rectus} and \textit{P. bifurcatus}. The diversity of morphotypes of \textit{P. arcuatus} could be due to the great abundance of identified materials of this ichnotaxon, since of \textit{P. rectus} and \textit{P. bifurcatus} there is only one representative of each. The greatest variation in the shape of the oviposition traces corresponded to the shape close to the apex, and to a lesser extent to the curvature and convexity of the trace. These three variations are also observed in the morphology of current Zygoptera eggs (manuscript in preparation).

On the other hand, no particular morphotype was observed for each oviposition substrate. Regardless of the substrate, the average shape of the traces does not vary. Therefore, we interpreted that the substrate would not be exerting shape modification pressures on the oviposition traces of the studied materials. In addition, a marked overlap was observed in the shape of the traces regardless of their locality of origin (LH - Lower Eocene - and RP - Middle Eocene -), so it could be inferred that the shape of the individual traces would not depend on provenance or age. Therefore, the variations in the shape of the individual traces would not be related to the ichnotaxonomy, substrate used or locality of provenance or age.

In summary, the shapes of Odonata’s individual egg traces are observed to be stable over 4 million years (Lower Eocene-Middle Eocene), while individual size varies. Insect eggs exhibit a great diversity of sizes, these variations are observed within populations (17, 18) and there are even variations in the eggs that the same female oviposes [e.g. (19)]. Therefore egg size results in a highly variable parameter that is also observed in the traces of Patagonia Odonata (Lower-Middle Eocene). On the other hand, in this work no consistent changes in egg shape are observed despite the different substrates used for oviposition, the 4 million years considered in this study and this is not reflected by the ichnotaxonomy either. According to Legay (15) insects have existed for hundreds of millions of years, but despite their great diversification, the shape of their eggs shows great stability. As far as we know, this is the first work that analyzes the change of shape of Odonata’s endophytic eggs in a scale of 4 million years and therefore, it would be the first work that empirically
reflects the stability of shape proposed by Legay (15). This could reflect that the shape of Odonata eggs, unlike their size, could have a strong evolutionary constrain already observed since the Patagonian Eocene.

Data accessibility. Our data are deposited at Dryad: https://doi.org/10.5061/dryad.d51c5b015. Dryad review URL https://datadryad.org/stash/share/VFMIA6wzw5HFORolcfipntAxkOMQtX0dUuC5319sJJ4.

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Supporting information labels:

Appendix S1. Material MPEF-IC-1382. A) Original material; B) Partial photographs taken with the magnifying glass completing the whole sheet. It has an overlapping layer in which the contours of each trace were marked and identified with a number; C) Detail of the layer with marked and identified traces; D) Principal component analysis graph PC 1 (59.41 %) - PC 2 (32.41 %) with 95% confidence ellipse. Scales: A) and B) 1 cm, C) 1x1 mm black square.

Appendix S2. Material MEF-IC-1376. A) Original material; B) Partial photographs taken with a magnifying glass completing the whole leaf. It has an overlapping layer in which the contours of each trace were marked and identified with a number; C) Detail of the layer with marked
and identified traces; D) Principal component analysis graph PC 1 (60.06 %) - PC 2 (30.30 %) with 95% confidence ellipse. Scales: A) and B) 1 cm, C) 1x1 mm black square.

Appendix S3. Material MEF-IC-1385. A) Original material; B) Partial photographs taken with a magnifying glass completing the whole leaf. It has an overlapping layer in which the contours of each trace were marked, which were identified with a number; C) Detail of the layer with marked and identified traces; D) Principal component analysis graph PC 1 (87.44 %) - PC 2 (7.55 %) with 95% confidence ellipse. Scales: A) and B) 1 cm, C) 1x1 mm black square.

Appendix S4. Material MEFP-IC-1388. A) Original material; B) Partial photographs taken with a magnifying glass completing the whole leaf. It has an overlapping layer in which the contours of each trace were marked, which were identified with a number; C) Detail of the layer with marked and identified traces; D) Principal component analysis graph PC 1 (57.06 %) - PC 2 (23.80 %) with 95% confidence ellipse. Outliers traces numbers: 31, 33, 40, 95, 98 and 240. Scales: 1) and 2) 1 cm, 3) 1x1 mm black square.

Appendix S5. Material MEF-IC-1378. A) Original material; B) Partial photographs taken with a magnifying glass completing the whole leaf. It has an overlapping layer in which the contours of each trace were marked, which were identified with a number; C) Detail of the layer with marked and identified traces; D) Principal component analysis graph PC 1 (55.01 %) - CP 2 (27.10 %) with 95% confidence ellipse. Outliers trace numbers: 45, 48, 50, 54, 71 and 72. Scales: A) and B) 1 cm, C) 1x1 mm black square.
Figure 1. A) Image of the Dicotyledonous leaf with traces of Odonata endophytic eggs located along the entire leaf surface (MPEF-IC-1388), scale: 1 cm. B) Superposition of layers to the main photograph of the leaf in which the contours of each trace were marked in detail. C) Detail of an egg trace with the scale (square 1 x 1 mm) in the lower margin. D) Map of Argentina showing the localities of Río Pichileufú and Laguna del Hunco in the Patagonia.

165x160mm (300 x 300 DPI)
Figure 2. Classical morphometrics of the traces of Odonata endophytic oviposition in relation with their ichnotaxonomy (A-E) and for Paleoovoidus arcuatus, in relation with the age/locality (F-J). Mean values + standard errors are displayed. Different letters between columns indicate statistically significant differences (p < 0.05).

165x200mm (300 x 300 DPI)
Figure 3. Classical morphometrics of the oviposition traces of Odonata in relation with the substrate used. Average + standard error shown. Different letters between columns indicate statistically significant differences ($p < 0.05$).

165x282mm (300 x 300 DPI)
Figure 4. Variation of shape in the traces of oviposition in fossil leaves from Laguna del Hunco and Río Pichileufú according to ichnotaxon. MPEF-IC 1376 Paleoovoidus rectus (Laguna del Hunco); MPEF-IC 1386 Paleoovoidus bifurcatus (Laguna del Hunco); MPEF-IC 1388 Paleoovoidus arcuatus (Río Pichileufú); MPEF-IC 1378 Paleoovoidus arcuatus (Laguna del Hunco). The variation in shape is represented in standard deviation units (-2 and +2) and the mean shape is the mean of the Fourier coefficients for all forms analysed.

165x94mm (300 x 300 DPI)
Figure 5. Analysis of the principal components of Fourier coefficients, of the individual traces analysed in 25 samples in relation to the 3 identified ichnotaxa (black dots correspond to MPEF-IC-1382 material assigned to P. arcuatus).

165x88mm (300 x 300 DPI)
Figure 6. Analysis of the principal components of Fourier coefficients, of the individual traces analysed in relation to the 12 oviposition substrates identified at family or species level.

165x88mm (300 x 300 DPI)
Figure 7. Analysis of the principal components of the Fourier coefficients, of the individual traces analysed in 25 samples in relation to their origin: Laguna del Hunco (Lower Eocene) and Río Pichileufú (Middle Eocene).
Table 1. Materials analysed: identification, locality (RP = Río Pichileufú; LH = Laguna del Hunco), paleobotanical classification, number of traces and measurements (Average ± Standard Error). Values are expressed in mm. P. = Paleoovoidus

| MPEF-IC | Location | Substrate       | Ichnospecies   |
|---------|----------|-----------------|----------------|
| 1386    | RP       | Dicotyledonous  | P. arcuatus    |
| 1388    | RP       | Dicotyledonous  | P. arcuatus    |
| 1390    | RP       | Dicotyledonous  | P. arcuatus    |
| 1391    | RP       | Dicotyledonous  | P. arcuatus    |
| 1393    | RP       | Dicotyledonous  | P. arcuatus    |
| 1382    | LH       | Dicotyledonous  | P. arcuatus    |
| 1367    | LH       | Dicotyledonous  | P. arcuatus    |
| 1371    | LH       | Dicotyledonous  | P. arcuatus    |
| 1372    | LH       | Dicotyledonous  | P. arcuatus    |
| 1375    | LH       | Dicotyledonous  | P. arcuatus    |
| 1380    | LH       | Dicotyledonous  | P. arcuatus    |
| 1383    | LH       | Dicotyledonous  | P. arcuatus    |
| 1384    | LH       | Dicotyledonous  | P. arcuatus    |
| 1381    | LH       | Malvaceae       | P. arcuatus    |
| 1370    | LH       | Celtidaceae     | Celtis ameghenoi | P. arcuatus |
| 1374    | LH       | Cunoniaceae     | Cupania latifolioides | P. arcuatus |
| 1377    | LH       | Fabaceae        | Cassia argentinensis | P. arcuatus |
| 1378    | LH       | Proteaceae      | Lomatia occidentalis | P. arcuatus |
| 1389    | LH       | Sapindaceous    | Schmidelia proedulis | P. bifurcatus |
| 1385    | LH       | Myrtaceae       | Myrcia deltodea | P. arcuatus |
| 1368    | LH       | Eucalyptus chubutensis | P. arcuatus |
| 1373    | LH       | Eucalyptus chubutensis | P. arcuatus |
| 1376    | LH       | Eucalyptus chubutensis | P. rectus |
| 1392    | LH       | Eucalyptus chubutensis | P. arcuatus |
| n traces | Length (L) | Width (W) | L/W Index | Area (mm²) | Perimeter |
|----------|------------|-----------|-----------|------------|-----------|
| 8        | 1.28 ± 0.04| 0.77 ± 0.02| 1.67 ± 0.05| 0.75 ± 0.03| 3.47 ± 0.08|
| 240      | 0.87 ± 0.01| 0.33 ± 0.01| 2.75 ± 0.03| 0.21 ± 0.01| 2.02 ± 0.04|
| 126      | 1.21 ± 0.01| 0.46 ± 0.01| 2.65 ± 0.03| 0.48 ± 0.01| 3.09 ± 0.03|
| 58       | 0.81 ± 0.02| 0.29 ± 0.01| 2.89 ± 0.07| 0.17 ± 0.01| 1.90 ± 0.05|
| 44       | 1.34 ± 0.02| 0.55 ± 0.01| 2.45 ± 0.05| 0.54 ± 0.02| 3.32 ± 0.05|
| 18       | 0.99 ± 0.04| 0.41 ± 0.02| 2.44 ± 0.04| 0.32 ± 0.03| 2.45 ± 0.11|
| 294      | 0.95 ± 0.01| 0.35 ± 0.35| 2.82 ± 0.03| 0.26 ± 0.01| 2.31 ± 0.03|
| 30       | 0.91 ± 0.02| 0.31 ± 0.01| 2.95 ± 0.08| 0.22 ± 0.01| 2.19 ± 0.04|
| 21       | 1.18 ± 0.02| 0.52 ± 0.01| 2.29 ± 0.05| 0.50 ± 0.02| 3.05 ± 0.05|
| 36       | 1.12 ± 0.03| 0.38 ± 0.02| 3.00 ± 0.10| 0.33 ± 0.02| 2.68 ± 0.08|
| 31       | 0.91 ± 0.03| 0.33 ± 0.01| 2.84 ± 0.09| 0.19 ± 0.01| 2.09 ± 0.08|
| 39       | 1.16 ± 0.04| 0.40 ± 0.02| 2.97 ± 0.09| 0.37 ± 0.03| 2.78 ± 0.10|
| 21       | 1.07 ± 0.05| 0.47 ± 0.03| 2.36 ± 0.09| 0.39 ± 0.04| 2.66 ± 0.14|
| 5        | 0.79 ± 0.10| 0.25 ± 0.03| 3.12 ± 0.14| 0.15 ± 0.03| 1.83 ± 0.23|
| 65       | 1.35 ± 0.02| 0.47 ± 3E⁻³| 2.85 ± 0.04| 0.49 ± 0.01| 3.26 ± 0.03|
| 38       | 1.00 ± 0.02| 0.34 ± 0.01| 3.06 ± 0.10| 0.26 ± 0.01| 2.36 ± 0.04|
| 30       | 0.98 ± 0.03| 0.35 ± 0.01| 2.79 ± 0.08| 0.25 ± 0.01| 2.33 ± 0.06|
| 84       | 1.10 ± 0.01| 0.42 ± 4E⁻³| 2.63 ± 0.03| 0.36 ± 0.01| 2.71 ± 0.03|
| 49       | 0.77 ± 0.02| 0.32 ± 0.01| 2.44 ± 0.06| 0.17 ± 0.01| 1.84 ± 0.05|
| 6        | 1.75 ± 0.13| 0.62 ± 0.03| 2.86 ± 0.28| 0.86 ± 0.08| 4.27 ± 0.26|
| 46       | 0.84 ± 0.02| 0.41 ± 0.01| 2.11 ± 0.06| 0.24 ± 0.01| 2.07 ± 0.06|
| 4        | 0.79 ± 0.08| 0.33 ± 0.02| 2.49 ± 0.37| 0.19 ± 0.02| 1.94 ± 0.16|
| 28       | 0.84 ± 0.04| 0.35 ± 0.02| 2.51 ± 0.10| 0.22 ± 0.02| 2.05 ± 0.10|
| 9        | 0.80 ± 0.05| 0.31 ± 0.02| 2.63 ± 0.19| 0.15 ± 0.02| 1.75 ± 0.11|
| 16       | 0.84 ± 0.05| 0.23 ± 0.01| 3.75 ± 0.20| 0.12 ± 0.01| 1.80 ± 0.10|
Table 1. Materials analysed: identification, locality (RP = Río Pichileufú; LH = Laguna del Hunco), paleobotanical and measurements (Average ± Standard Error). Values are expressed in mm. P. = P
Table 1. Materials analysed: identification, locality (RP = Río Pichileufú; LH = Laguna del Hunco), paleobotanical ... classification, number of traces and measurements (Average ± Standard Error). Values are expressed in mm. P. =

*Paleoovoidus*
Table 2. Summary table of values of the Principal Components (PC) of the Fourier coefficients.

| PC  | n   | Mean | S.E. | Min  | Max  | Median |
|-----|-----|------|------|------|------|--------|
| PC 1| 25  | 62.87| 2.39 | 43.33| 98.81| 60.06  |
| PC 2| 25  | 22.71| 1.62 | 0.73 | 36.32| 23.8   |
| PC 3| 25  | 7.3  | 0.69 | 0.46 | 14.86| 6.89   |
coefficients.
Associate Editor: Dr. Jeffrey Thompson  
Subject Editor: Kevin Padian  
Editorial Coordinator: Lianne Parkhouse  
Royal Society Open Science  

Dear Editors  

Thank you for considering our manuscript “GEOMETRIC MORPHOMETRICS OF ENDOPHYTIC OVIPOSITION TRACES OF ODONATA (EOCENE, ARGENTINA)” for Royal Society Open Science, and for the opportunity to submit a revised version. We are grateful for all the constructive comments and recommendations from the editor and reviewers, which helped us to improve the quality of the article.

We have made corrections taking into consideration all the comments received. Language and tables were carefully revised to improve the clarity of the presentation.

Below please find our point-by-point responses to the comments received and the changes we have made in the manuscript. The changes were also highlighted in yellow in the manuscript to facilitate the review.

If you need any further clarifications or changes please do not hesitate to contact us.  
Looking forward to your response,  
Best regards,

Dr. Eugenia Romero-Lebrón (on behalf of all coauthors)
Associate Editor Comments to Author (Dr Jeffrey Thompson):

Dear Eugenia et al,

Your manuscript has been reviewed by three expert reviewers, and I am happy to say that they have all been very positive about your manuscript. I am thus recommending acceptance with minor revisions. There are a few cases where things are not clear in the manuscript, where things are misspelled, or where the English could be improved, which has been highlighted by the reviewers in their attached, annotated, files. There are also a number of stylistic errors found in the references. I thus suggest you go through the manuscript with a fine-toothed-comb to correct these errors in spelling, formatting, and clarity prior to resubmission. Please address all suggestions and comments made by the reviewers prior to resubmission.

All the best,
Jeff Thompson

Re: Thank you for your positive words and for the opportunity to revise and resubmit our work.

Reviewer 1:

I included some corrections and comments in the pdf file of the manuscript (attached)

Re: Thank you for your constructive comments. We appreciate your detailed review.

Point-by-point response:

1. Line 15: authors consider these traces sometimes as scars that surrounds the eggs (traces) as in the first part of the abstract and several parts of the main text BUT also as simple eggs as in the end of the abstract and the end of the Discussion section. These are traces and not eggs thus along the manuscript the authors must argued and consider only the correct expression and definition
   Re: We appreciate this observation. The manuscript has been completely revised for conciseness.

2. Line 49: confusion: it seems authors indicate the current study, but clearly is not the case
   Re: Writing was revised for clarity.

3. Line 52: several times in the manuscript the spelling of this word is not correct: ichnospecies
   Re: Done

4. Line 75: to delete this comma
   Re: Done
5. Line 87: several times in the manuscript the spelling of this word is not correct: morphometrics
   Re: Done
6. Line 164 and 171: surely better using letters
   Re: Done
7. Line 174: Romera-Lebrón et al. Please, review the entire manuscript to check this kind of errata
   Re: We apologize for missing this typo, now thoroughly revised, which was related to some issues with the reference manager.
8. Line 174: change “analyse” to “analysed”
   Re: Done
9. Line 234 to 236: not clear in each case if the authors refer to extant behaviour or past behaviour or both. In any case, the sentence is confuse
   Re: We referred to behaviour inferred from the fossil record. The text was revised for clarity. It now reads: “… in the fossil record Lestidae females lay their eggs in specific substrates such as Daphnogene leaves (Lauraceae), while Coenagrionidae females do not appear to be selective.” (line 244 to 246)
10. Line 272: change “icnhnotaxa” to “ichnotaxon”
    Re: Done
11. Line 272: before the word ichnotaxon
    Re: Done
12. Line 272: Change “Rio” to “Río”. Please, revise the entire manuscript to eliminate this kind of errata
    Re: Done
13. Line 281: are, not were, because these are fossil ichnotaxa and originally were ovipositions (no fossils)
    Re: Done
14. Line 281: Change “presented” to “present”
    Re: Done
15. Line 282: Change “one” to “the same”
    Re: Done
16. Line 319: Change “current” to “extant”
    Re: Done
17. Line 322: Change “interpreted” to “interprete”
    Re: Done
18. Line 331: two sentences that must be separated using . or ;
    Re: Done
19. Line 334: Change “Patagonia” to “Patagonian”
    Re: Done
20. Line 354: “Rodrigues” maybe it is Rodríguez?
    Re: We appreciate the observation and agree that the surname Rodriguez is more usual, but in this case, his surname is Rodrigues (with “s”)
21. Line 362 to 379: comments on these 6 first references showing the need of a detailed review of all the references in the list:
   1- pages separated by different symbols.
   2- names of taxa not in italics.
   3- incorrect spelling of the name of one author: Petrulevicius to Petrulevičius.
   4- incorrect spelling of the word Fossillagerstatte.
   5- in ref 6 there is not; after the year of publication...
   Re: The reference list was carefully revised and corrected.

22. Line 548 and 549: S2-S5 not available to review
   Re: We hope they are available in the resubmission.

23. Figure 1: to include in this part of the figure caption both the taxon and the locality (and age).
   Re: Done

24. Figure 2: Change “Paleoovoidus arcuatus” to “Paleoovoidus arcuatus” in italics.
    To revise the entire manuscript
   Re: There was a format change when we logged the files. We hope it is corrected now.

25. Table 1: very confuse. The genus Lomatia is both Fabaceae and Proteaceae in this table. Please, revise carefully the two tables
   Re: The name was written using a lower alignment, thank you for noticing.
    Lomatia is a Proteaceae. We made the necessary corrections in the table.

26. Table 2: why only one decimal in this measure if the rest (except to 7.3) have two decimals?
    Re: Done

Reviewer 2:

The authors have attempted to study the shape variation of the egg traces of Odonata from the Eocene in Argentina, the article sound very original and the use of elliptical analyses of Fourier are indeed interesting. I missed a more sophisticated analysis implemented in the package “Momocs” for Fourier analyses, the use of old implementation is not bad but always can be better.

I have some comments written in the manuscript PDF please take a look all of them mostly are of form, but I have some principal concern regarding the substrate variation, the authors said very explicit that there are no pattern related, but I noticed at least 3 pattern of 3 different substrate in the PCA, from Eucalyptus chubutensis (very clear variation in mostly all the morphospace) and in Malvaceae (very narrowed group of specimens not superposed to the others).

Nevertheless, I like the idea and I’m sure that the article can be accepted after minor revision comments of the PDF and improve the manuscript
Re: We appreciate your positive words and your detailed review. We also appreciate your suggestion and will use the "Momocs" package in our future research.
In relation to the 3 patterns you mention, we have revised/improved our interpretation accordingly. Please see response to point 16 below.

Point-by-point response:

1. Line 29: Change “4” to “four”
   Re: Done
2. Line 30: “Odonata” order? or general, odonates
   Re: In this sentence we refer to the sexual behaviour of the order Odonata, since it is the only extant order of Odonatoptera and therefore the only order from which we can know precisely their sexual behaviour.
3. Line 80: what is a CCD?
   Re: CCD is the charged-coupled device, which is a transistorized light sensor on an integrated circuit. We now state what the acronym stands for
4. Line 85: better modify to traditional morphometrics is more accurate than classical
   Re: We agree
5. Line 151: This information belong to the Methods
   Re: Done
6. Line 171: decide 1 or one
   Re: One. Done
7. Line 182, 183 and 187: not clear what is wider? Greater?
   Re: This paragraph was revised for clarity.
8. Line 183: “(p < 0.05)” which test provide this significance?
   Re: Comparisons were made using Generalized and Mixed Linear Models (MLGM) and LSD Fisher as a posteriori test (Alpha = 0.05). This is described in the Materials and Methods section (line 109 and 140). We have revised the paragraph for clarity.
9. Line 189: what is relatively little?
   Re: By “little variations” we meant that the variations observed are not statistically significant; this is now clearly stated.
10. Line 193: you performed 25 PCAs?
    Re: Yes, for each individual substrate we performed a principal component analysis. We have submitted a figure for each material with the contours of each trace and the PCAs that I hope will be available in the electronic version. If not, appendices S1-S5 show the PCAs of 5 materials (MPEF-IC-1382, MPEF-IC-1376, MPEF-IC-1385 and MPEF-IC-1388).
11. Line 194: The figure 4 not correspond to a PCA, correspond to the shape variation, please modify.
    Re: Yes, excuse the mixup. We appreciate the observation and have corrected the text accordingly.
12. Line 195: I don't understand this, how a single PC can explain a range of % of shape variation, here the author need to be more specific...
   Re: We realize the text was confusing and the paragraph was rewritten. We did not imply that a single PC explained a range of % variation; instead, we referred to the range of variation explained when considering the 25 PCA we carried out (one for each substrate). We have revised the text for clarity. We also replaced Table 2, which was meant as an overall summary, with the PCA for each of the 25 substrates.

13. Line 196: not clear what are you saying here.
   Re: Component 1 mainly discriminated the shape of the apex of the trace. The text was rewritten.

14. Line 204: Change “total variation observed” to “total shape variation”
   Re: Done.

15. Line 211: Change “in relation to” to “related to”
   Re: Done

16. Line 216: I disagree with the authors in this observation, Malvaceae is indeed very different to the other groups, and the Eucalyptus chubutensis and Myrcia deltodea also have some important tendency
   Re: We agree, you are right and we appreciate this observation. We have improved the description of the figure in the text and its discussion. It is true that Malvaceae is different from other substrates, but we prefer to take this result very carefully. To draw robust conclusions from this family is complicated as there is only 1 material which has only 5 traces (Table 1) (please see the paragraph starting on line 223 of the results section and then line 336 of the discussion section).

17. Line 221: reduced the morphospace to only 3 PC's? not clear what is reduced here...
   Re: The text was corrected. The first three components together explain nearly 93% of shape variability.

18. Line 321: At least Malvaceae was very clear, please explain why this substrate could be different to the others... Also explain why the shape variance of Eucalyptus chubutensis is higher than all the other substrate
   Re: As we explained in point 16 you are right to note the pattern in the Malvaceae family, and this in now mentioned in the manuscript. But because of the N (1 specimen with only 5 traces) we chose to be careful in drawing conclusions about the Malvaceae family. On the other hand, Eucalyptus chubutensis showed the widest morphospace, which in part could be explained by the larger number of separate samples (N = 4) analysed (see Figure 6 B in revised version). However, Myrcia deltodea also shows a wide morphospace, with all traces coming from the same substrate material. Therefore, we interpret that the substrate would not be exerting differential shape modification pressures on the egg traces between the studied materials.
Reviewer 3:

Dear Authors,

Congrats for your manuscript. There is tiny mistakes to be corrected with english and one sentence in the discussion that seems awkward to me.

Otherwise, manuscript deserves to be publish as is.

Illustration and the effort of represent each oviposition per leaf in a draw are very good.

Re: Thank you for your positive words and constructive comments.

Point-by-point response:

1. Line 171: I don't understand the end of this sentence. What do you mean by "unidentified" followed by "now assigned to"?
   Re: Text was revised for clarity; we meant that we assigned the specimen to the ichnoclass.

2. Line 284 and 296: Change “ichnospecie” to “ichnospecies”
   Re: Done

3. Line 339: I do not appreciate this sentence despite the fact I understand what do you mean. It look akward to write "as far as we know" in a scientific publication or not. This sentence looks like to say: according our personal memory, there is no study. No, you should demonstrate in this sentence that you search on the litterature and there is nothing. ☹
   Re: We appreciate the observation. We modified the text to make it clear that we searched and did not find any literature on the subject. ☺