

Another one bites the dust: A new *Lithoserix* species (Hymenoptera, Ichneumonidae, Pimplinae) from the early Oligocene in France, with an evaluation of wing morphometrics

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Abstract

A new Darwin wasp species, *Lithoserix oublieri* **sp. nov.** is described and illustrated from the early Oligocene limestone formation Calcaires de Campagne-Calavon in the Luberon Region, France. It represents the third species of this extinct genus, which was first described from the late Eocene Florissant Formation in Colorado, US and later found in Aix-en-Provence, France, from the late Oligocene. The taxonomic placement of this genus in the context of tribal classification is analysed and discussed, based on geometric morphometrics of the fore and hind wing venation of fossil and extant Pimplinae species. The results suggest that *Lithoserix* does not belong to the same group as the extinct genus *Crusopimpla*, but rather represents a more basal genus within Pimplini or belongs to an extinct separate tribe, closely related to Pimplini.

Key Words

Calcaires de Campagne-Calavon Formation, compression fossils, Darwin wasps, fossil record, geometric morphometrics

Introduction

Pimplinae is a species-rich subfamily of Darwin wasps that began to diversify in the Cretaceous (Kopylov 2009; Kopylov et al. 2010; Spasojevic et al. 2021). Currently, Pimplinae is regarded as paraphyletic (Spasojevic et al. 2021) and consists of four tribes. Pimplini, the tribe that branches off first, is much older than the other pimpline tribes and most other subfamilies in the informal group Pimpliformes (except Diplazontinae). The other three tribes group together, with Ephialtini branching off first, resulting in Delomeristini and the recently resurrected Theroniini being sister groups (Klopfstein et al. 2018). Notably, while no extinct tribes exist in Pimplinae, there are some extinct genera, such as *Crusopimpla*, which was first described from the Tadushi Formation in Russia (Eocene) (Kopylov et al. 2018) and later found in the Fur Formation in Denmark (Eocene) (Klopfstein 2022). Another extinct

pimpline genus is *Lithoserix*, which was first described from the late Eocene Florissant Formation in Colorado (Brown 1986). It was originally placed in Siricidae, but later moved to Ichneumonidae (Kasparyan and Rasnitsyn 1992). The second species of *Lithoserix*, *L. antiquus* (Saussure, 1852), was found in Aix-en-Provence in France (late Oligocene) and only transferred to the genus recently (Spasojevic et al. 2022). Within Darwin wasps, *Lithoserix* was initially placed in Ephialtini by Kasparyan and Rasnitsyn (1992); however, this placement was questioned due to its resemblances to some Delomeristini and Theroniini, as well as to *Xanthopimpla* (Spasojevic et al. 2022). Moreover, many important body characters are not preserved in the *Lithoserix* fossils, making their tribal placement even more difficult (Spasojevic et al. 2022).

In contrast to body characters, which often exhibit varying degrees of preservation in compression fossils, wings and their venation are often consistently well-preserved, making

them an important character system for identifying fossils. Moreover, recent studies have demonstrated that fore wing characteristics can be used to distinguish different subfamilies of Darwin wasps (Li et al. 2020; Meier et al. 2022; Viertler et al. 2022). Within Pimplinae, some tribes are known to possess specific hind wing vein characteristics, such as the interception of the nervellus, which is clearly above the middle in Pimplini, while in Ephialtini, it is often around or below the middle (Gauld et al. 2002). However, it can also be clearly above in quite a few Ephialtini such as *Dolichomitus*, *Ephialtes* etc.

The newly-described fossil species is from the Calcaires de Campagne-Calavon Formation in the Luberon Region of south-eastern France. This formation is around 31–30 million years old (early Oligocene) and includes numerous fossiliferous localities (Ducieux et al. 1985; Duhamel and Louchart 2020; Coster and Legal 2021). Its sedimentary deposits consist mostly of laminated limestones (Coster and Legal 2021) and its paleoenvironment is considered a quiet, calm and shallow lacustrine setting (Duhamel and Louchart 2020). The formation is known for its rich fossil finds, ranging from birds to fish, insects and plant remains (Duhamel and Louchart 2020; Coster and Legal 2021). Most fossil insects are Coleoptera and Diptera (Skartveit and Nel 2017; Nel et al. 2023), while descriptions of Darwin wasps from this location are rather scarce. There is one major publication by Nicolas Théobald (1937), providing an overview of insects in Oligocene formations of France, including two described Darwin wasps (*Pimpla aquensis* Théobald, 1937 and *P. anomalensis* Théobald, 1937) found in Céreste, a locality of Calcaires de Campagne-Calavon. However, *P. anomalensis* probably does not belong to Pimplinae since the areolet seems pentagonal, a feature not found in Pimplinae. Additionally, *P. aquensis* is difficult to assign, but it appears to have a rather petiolate or strongly tapering first tergite, which would be uncommon in Pimplinae.

In this study, I describe and illustrate the third species of the extinct genus *Lithoserix*. Based on a geometric morphometric analysis of wing venation, including extant and fossil species of the four pimpline tribes and the unplaced fossil genus *Crusopimpla*, I discuss the taxonomic placement of *Lithoserix*.

Materials and methods

Fossil material

The fossil specimen (PNRL-SIG-216, Signoret collection of the Parc naturel régional du Luberon (PNRL)) is from the Calcaires de Campagne-Calavon Formation. The exact provenance of the locality is unknown.

Photos were taken with a Keyence VHX 600 camera system with a magnification of 50–200. Measurements were then taken with ImageJ (Abramoff et al. 2004). Using different photos of the specimen as templates, I made interpretative drawings of the fossil in Adobe Photoshop (ver. 25.1.0). Uncertainties of fossil structures are indicated

by dotted lines. Terminology mainly follows Broad et al. (2018), with the exception of the wing venation, which follows Spasojevic et al. (2018). Tergites are abbreviated as “T1”, “T2” etc. For the comparison of the fossil with other taxa, I used identification keys and diagnostic characters from several taxonomic treatments (Townes 1969; Kasparyan and Rasnitsyn 1992; Spasojevic et al. 2022).

The colours of fossils may change due to preservation, requiring consistent patterns for clear interpretation. Colour and colour pattern preservation in ichneumonid fossils were evaluated before and remarkable consistency was found in various holotypes and its paratypes, as well as in parts and counterparts (Klopfstein 2022). Unfortunately, the fossil specimen in this study has no counterpart and there were no paratypes, which complicates confirming colour alterations. Nevertheless, if the new fossil species displays consistent colouration on both body sides (e.g. right and left hind femur), the interpretation is noted in the species description, but should be treated with caution.

Geometric morphometrics

The landmark dataset of the fore wing encompasses 62 Pimplinae taxa from Viertler et al. (2022), representing all four tribes with 49 of the 79 known genera, plus 10 fossil species. I added the wing venation of the two previously described *Lithoserix* species (see Suppl. material 1 for taxon list), plus of the new species described herein. For the fore wing, 20 fixed landmarks are included in this analysis (Fig. 1A). Viertler et al. (2022) used 21 fixed landmarks, but the location of the first landmark is not certain in the new fossil species and is removed here. The dataset covers all four pimpline tribes, as well as the extinct genera *Crusopimpla* and *Lithoserix*, which are both not placed within a pimpline tribe. Each tribe and each extinct genus are treated as a pimpline “group” in the wing analyses, of which there are six in total.

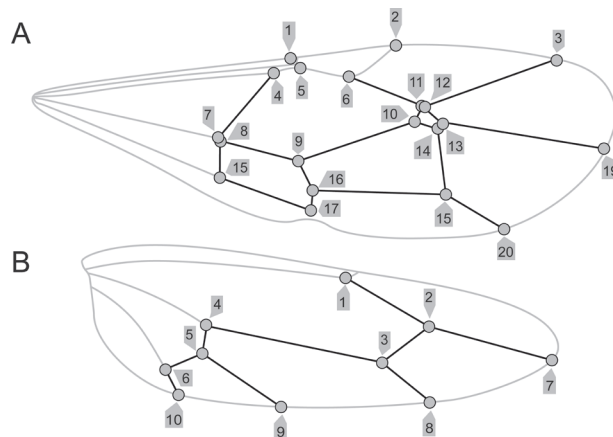


Figure 1. Landmark sets of fore- and hind wing. **A.** Schematic representation of a fore wing with 20 landmarks (grey circles) and **B.** hind wing with ten landmarks (grey circles) placed on the respective intersections of the veins. The black lines indicate the veins, which connect the landmarks.

As previously done in Viertler et al. (2022) for the fore wings, the illustrations of the hind wing from Townes (1969) were used to place ten fixed landmarks (Fig. 1B) with the software tpsDig2 (Rohlf 2008). Four *Crusopimpla* species (*C. collina* Klopstein, 2022, *C. elongata* Klopstein, 2022, *C. minuta* Klopstein, 2022 & *C. rettigi* Klopstein, 2022), *Theronia? nigriscutum* Klopstein, 2022 and *Lithoserix antiquus* were excluded from the hind-wing dataset, because some landmarks could not be placed due to incomplete hind-wing preservation. Additionally, three taxa, *Zatypota percontator* (Müller, 1776), *Sinarachna pallipes* (Holmgren, 1980) and *Eriostethus rufus* (Uchida, 1932), belonging to Ephialtini, were removed from the hind-wing dataset since their nervellus is not intercepted and, therefore, LM 5 and LM 9 could not be placed.

Differences in wing venation shape between the groups were evaluated and possible affiliations of *Lithoserix* and *Crusopimpla* to the four pimpline tribes investigated. First, a generalised Procrustes analysis was performed to scale, translate and rotate the landmark configurations using the function gpgen from geomorph (Adams et al. 2022) in R studio (ver. 4.0.2, RStudio Team 2020). To test how size and group affiliation interact with the wing shape, a Procrustes regression for the hind and fore wings was performed with procD.lm. This output was then used in the function plotAllometry, where first the standardised shape scores are calculated from the regression of shape on centroid size and plotted against centroid size. Then the same output is used to plot the predicted values of the first principal component against centroid size, showing the allometric trend per group (package geomorph, Adams et al. (2022)). Additionally, the Procrustes shape coordinates were used to perform a between-group Principal Component Analysis (bgPCA), where the variation of the groups (four pimpline tribes, *Crusopimpla*, and *Lithoserix*) was examined in a leaving-one-out crossvalidation (10,000 permutations) with the function groupPCA from the R package Morpho (Schlager 2017).

Further, a Canonical Variate Analysis (CVA) was conducted using the function CVA from Morpho (Schlager 2017) to gain additional information for evaluation of shape patterns (Renaud et al. 2015; Mennecart et al. 2020). The CVA was applied to the fore and hind wing to visualise the maximised amongst-group variance relative to the within group variance and to evaluate the similarity of the two extinct genera to the extant pimpline tribes.

All data in connection with the geometric morphometric analyses are provided in the Supplementary materials (Suppl. materials 2, 3).

Results

Systematic palaeontology

Order Hymenoptera Linnaeus, 1758
Family Ichneumonidae Latreille, 1802
Subfamily Pimplinae Wesmael, 1845

Genus *Lithoserix* Brown, 1986

Lithoserix oublierus Viertler, sp. nov.

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Fig. 2

Type material. Holotype (PNRL-SIG-216, female, part, no counterpart available).

Etymology. Oublierus - from the French word “oublier” (forgotten) because the fossil was in the collection for a long time under the label “wasp” and was overseen, until André Nel and Corentin Jouault saw the wing venation and identified it as a Darwin wasp. The name is dedicated to the possibly numerous Darwin wasp fossils that are overlooked in natural history collections.

Type locality. South-eastern France, Calcaires de Campagne-Calavon Formation (Rupelian, 31–30 Ma).

Systematic placement. Many characteristics indicate that the fossil belongs to Pimplinae: the stout and short T1 with a lateromedian carina present, a quadratic areolet in the fore wing, 2m-cu slightly bowed outwards and two bullae and a long 2R1 cell. Other strong arguments for this subfamily are found in the hind wing: a long 1Rs relatively to its short rs-m vein, as well as a nervellus that is intercepted clearly above the middle. While the ovipositor of this fossil is only weakly discernible, it appears to project posteriorly from the metasoma, providing further support for its placement in Pimplinae.

The fossil also shows some character combinations that are rare in Pimplinae, but that are found in the extinct genus *Lithoserix*: its rather extensive propodeum carination, the fore wing with an almost triangular areolet, a long 1Rs + M and a sinusoidal 4Rs vein, together with the lateromedian carina on T1 reaching to the posterior end.

Diagnosis. There are currently two *Lithoserix* species described, which are both preserved from the dorsal side, while the new species is preserved more laterally. The new fossil specimen differs from both *Lithoserix* by having the vein 1cu-a strongly postfurcal, with 1Cu about 3–4× longer than its width, having 3Cu much longer with twice the length of 2cu-a, 1Rs much longer than vein rs-m in the hind wing and by its narrower hind femurs (Table 1).

Additionally, the new fossil specimen differs from *L. antiquus* by having a nervellus that is intercepted very high up, not having smooth transverse bands on the hind margins of T2–T7 and its lateromedian carina on T1 reaching beyond the middle, maybe even until the posterior end, but this is difficult to interpret. Furthermore, *L. antiquus* has brightly-coloured legs (orange), whereas the new species appears to have dark legs.

Finally, the new fossil species differs from *L. williamsi* by having its antennal segments less stout, at least not in the basal segments. Furthermore, *L. williamsi* is around twice the body size and has strongly impressed notauli, whereas they are only weakly preserved in the new species.

The fossil specimen exhibits shallow notauli and weak pleural, lateral longitudinal and lateromedian carination on the propodeum. The fore wing has a strongly postfurcal nervulus, the nervellus in the hind wing is intercepted

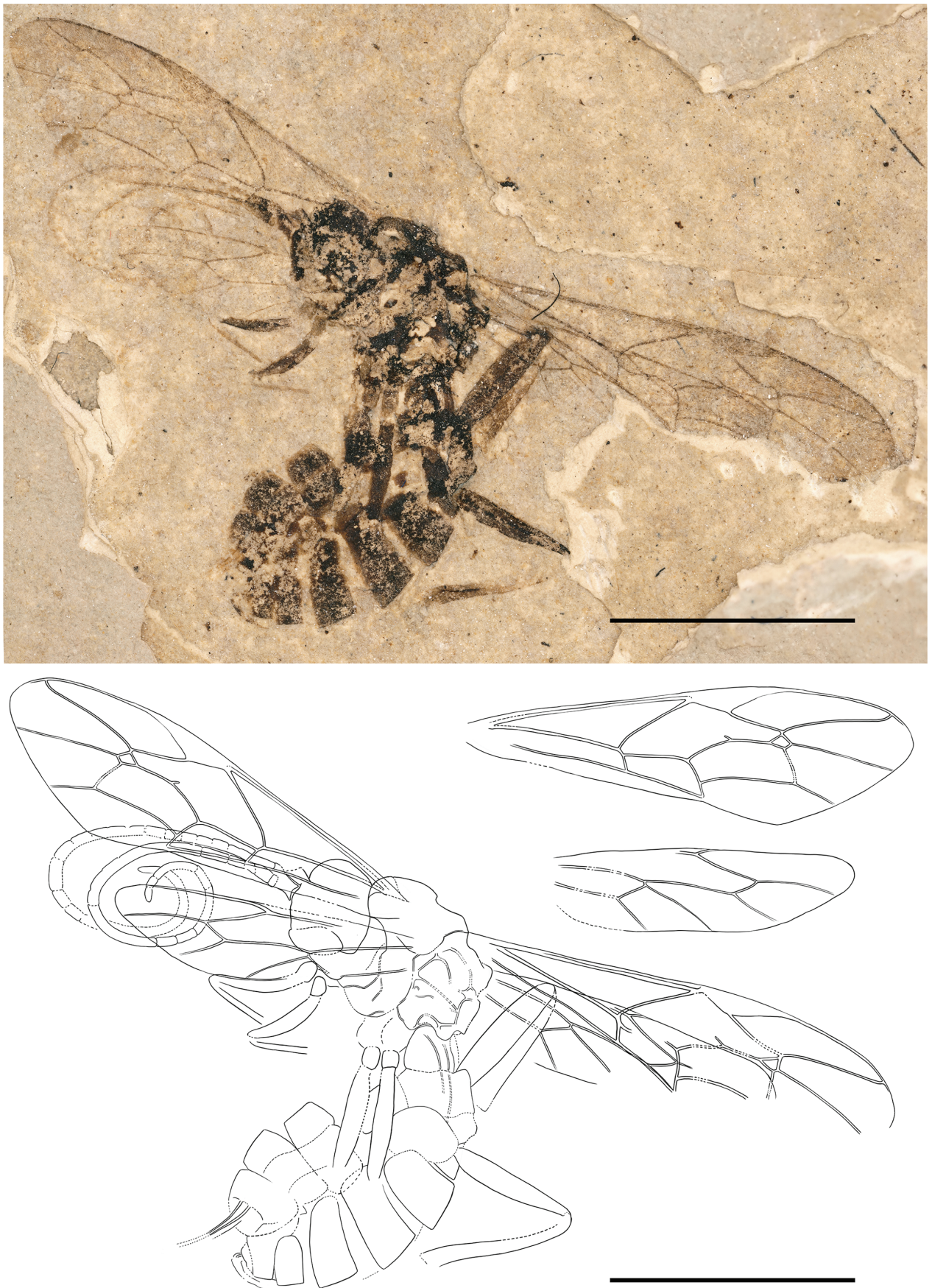


Figure 2. Holotype of *Lithoserix oublierus* sp. nov. (PNRL-SIG-216) **A.** Photograph of the holotype; **B.** Interpretative drawing, where dotted lines indicate uncertain interpretations. Scale bars: 5 mm.

in its anterior 0.2 and T1 features lateromedian carinae which reach beyond the middle. The fossil specimen's colouration is interpreted without having a counterpart or paratype, which would increase the certainty of the observed colours. However, the specimen shows both antennae and fore- and mid-coxae with a light colour, but a dark-coloured head, body and femurs. It appears that both hind tibiae have a bright base and a dark apex.

Table 1. Overview of differences between the three *Lithoserix* species.

Species	<i>L. williamsi</i>	<i>L. antiquus</i>	<i>L. oublieri</i>
Antennal segments, dimension	Short, only slightly longer than wide	?	1.5–2.5× longer than posteriorly wide
Mesoscutum, notauli	Extending past half of mesoscutum	Extending to basal third of mesoscutum	Weak or absent
Hind femur, dimension	3.4× longer than wide	3.5× longer than wide	4.2× longer than wide
Fore wing, 1cu-a meeting M+Cu	Interstitial	Interstitial	Strongly postfurcal
Fore wing, 3Cu length	1.3× 2cu-a	1× 2cu-a	2× 2cu-a
Fore wing, 4Rs shape	Clearly bowed at base	Little bowed at base and apex	Distally arched and slightly sinusoidal
Hind wing, nervellus	At anterior 0.3	At anterior 0.4	At anterior 0.2
Hind wing, 1Rs length	1.5× rs-m	1.3× rs-m	2.6× rs-m
T1, lateromedian carina	Beyond middle	Basal half	Beyond middle
Body length	22 mm	13.7 mm	12–13 mm

Description. Preservation. Holotype in dorso-lateral view. Antenna, head and mesosoma well preserved, but some details are missing or obscured by the very well-preserved fore- and hind wings. Legs are partially preserved, including all femora and fore- and hind tibiae, as well as fore- and mid-trochanters. Propodeal carination visible. Metasoma difficult to interpret since hind coxa and first tergite seem to overlap and the metasoma is preserved rather compressed, which is probably an artefact. Ovipositor partially visible at base, but otherwise indiscernible or broken.

Body. 12–13 mm. Fossil dark in colour, either black or dark brown. Antennae seem bright, but scape appears dark. All femurs dark, front tibiae appear bright. Hind tibiae appear bright with lower 0.4 dark.

Head. Antenna 10.8 mm, 1.1× fore wing length; without white band; dimensions of segments around 1.5–2.5× longer than posteriorly wide; number of antennal segments unclear, but more than 20; antenna more or less of even thickness throughout.

Mesosoma. Dimension unclear. Scutellum with shallow and slightly converging notauli. Metapleuron appears as long as wide, with juxtacoxal carina present. Propodeum rounded posteriorly; about as long as high; with small oval spiracle; traces of pleural carina, lateral longitudinal and lateromedian carina present, at least anteriorly and

posteriorly; posterior transverse carina present. Fore legs slender; hind femur 4.2× as long as wide.

Wings. Fore wing 9.7 mm. Areolet closed, slightly petiolate almost triangular, 2-Rs same length as 2-rs-m, 4M 1.1× 2-Rs and 2+3M 0.6× 2-Rs. 2m-cu present, slightly bowed to straight, with two bullae. 4Cu 2× 5Cu. 4Rs distally arched and slightly sinusoidal. 1Rs + M present, longer than width of surrounding veins. 1cu-a distal of 1M+1Rs by more than vein width. Pterostigma length 4.2× width, 0.6× vein 1R1. Cell 2R1 4.1× longer as wide. 5M vein tubular through entire length. 2Cu 0.8× 1M+1Rs, 1.17× r-rs. 1m-cu&2Rs+M vein straight or weakly arched or angled. 3Cu 1.8× 2cu-a. Hind wing with 1Cu very short, 0.15× cu-a. Veins 2Rs and 2Cu tubular through entire length. 2Rs 2.4× rs-m.

Metasoma. Dimension unclear, but stout in appearance. T1 broad and short, parallel-sided with slightly narrower base, with lateromedian carina more than half length of tergite. Dimension of T2 unclear, but appears transverse, as do T3–T6. Sternites strongly sclerotised, as dark as tergites. Ovipositor length unknown, but seems to reach past posterior end of metasoma.

Shape variation in Pimplinae explained by group and size

The regression of shape on centroid size accounted for 30.0% of the fore-wing shape variation in Pimplinae ($p = 0.001$) (Table 2). The remaining shape variation of the fore wing can be explained to 16.7% by the pimpline group affiliation (the four tribes and two extinct genera) ($p = 0.001$); however, no interaction between the two variables could be observed ($p = 0.218$). Hence, despite significant common allometry within groups, differences in shape amongst groups are still observed (Fig. 3A), but are similar for Ephialtini, Delomeristini and Theroniini and similar within Pimplini and *Lithoserix*.

In the hind wing, the shape is explained to 25.9% by the centroid size ($p = 0.001$) and 16.3% by the group affiliation ($p = 0.001$) (Table 2). The shape changes correlated with the interaction of centroid size and group affiliation by 10.3% ($p = 0.004$). Here, an interaction between both was observed ($p = 0.004$), indicating unique allometries in the different groups, while still showing differences in shape amongst tribes (Fig. 3B).

Pimpline group differences in fore wings

The bgPC1 of the fore wings explains 64.3% of the vein-shape variance associated with the groups, while bgPC2 explains 19.7% of the shape variation (Fig. 4). In those two bgPCA axes, the two extinct genera *Crusopimpla* and *Lithoserix* group separately from the extant tribes with low bgPC2 scores and also seem distinctly different from each other on the bgPC1 axis. The mean shape of *Crusopimpla* appears to have broader and shorter cells,

Table 2. Statistical results of Procrustes ANOVA for fore and hind wing. Df (Degree of freedom), (SS) Standard deviations of observed Sums of Squares, MS (mean squares), Rsq (R squares), F (F-value), Z (Z-score), Pr(>F) (*p*-value of F statistic).

<i>Fore wing</i>							
Effect	Df	SS	MS	Rsq	F	Z	Pr(>F)
Centroid size	1	0.16201	0.162014	0.30013	33.24	5.4689	0.001
Group	5	0.09048	0.018096	0.16762	3.713	5.5147	0.001
Centroid size: Group	5	0.02906	0.005813	0.05384	1.193	0.7919	0.218
Residuals	53	0.25825	0.004873	0.47841			
Total	64	0.53981					
<i>Hind wing</i>							
Centroid size	1	0.19919	0.199191	0.25963	26.8507	4.9981	0.001
Group	5	0.12550	0.025100	0.16358	3.3834	3.8758	0.001
Centroid size: Group	5	0.07901	0.015803	0.10299	2.1302	2.5668	0.004
Residuals	49	0.36351	0.007418	0.47380			
Total	60	0.75921					

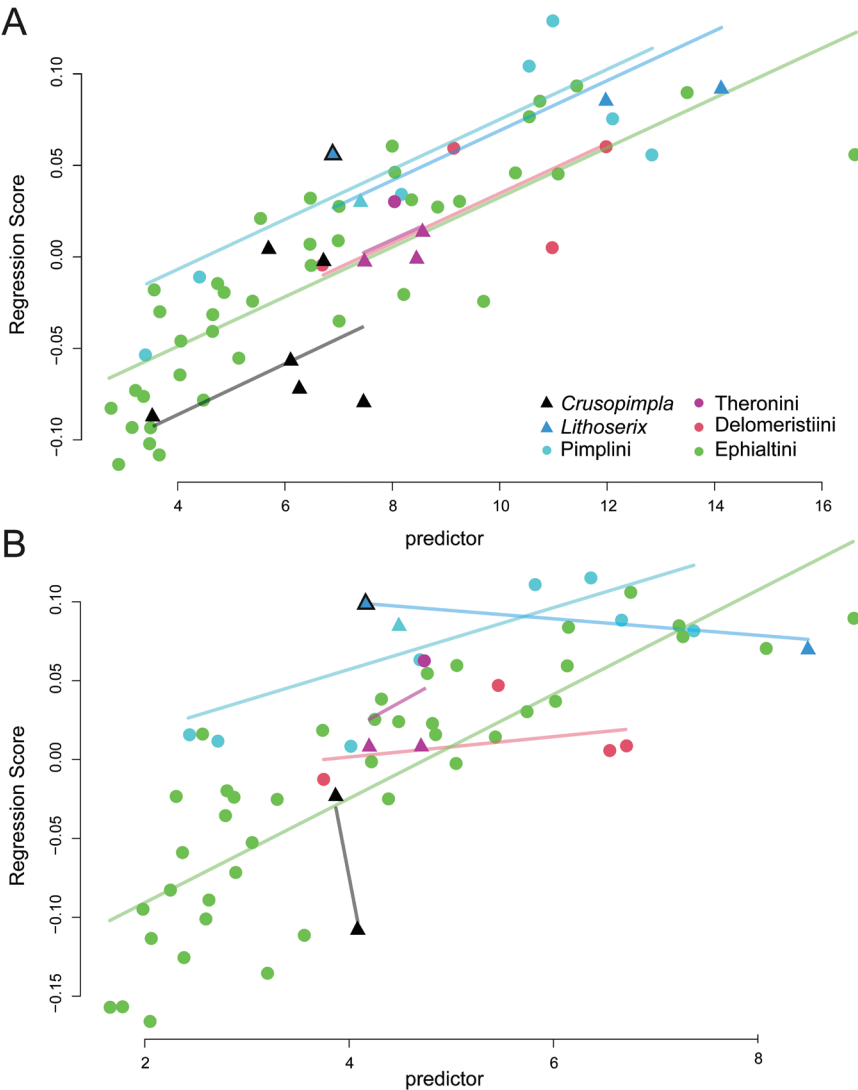


Figure 3. Regression of pimpline wings of shape on size (regression score) plotted against centroid size (predictor). Straight lines show the fitted values of PC1 against centroid size, indicating the allometric trend per tribe in **A**. Fore wing and **B**. Hind wing.

as well as a broad pterostigma and an elongated areolet with vein 3rs-m and 2+3M distinctly longer than 2Rs and 4M. Overall, the fore-wing shape of *Crusopimpla* differs highly significantly from most pimpline groups (Table 3). Additionally, the *Lithoserix* taxa have high bgPC1 scores and their mean shape is characterised by

an overall narrow wing with slender cells as seen in cells 2R1, 1M+1Rs and 2Cu and the areolet not as elongate and more located towards the distal margin of the wing. In addition, although *Lithoserix* does appear to group separately from the extant tribes (Fig. 4), it only shows distinct differences to Ephialtini (Table 3).

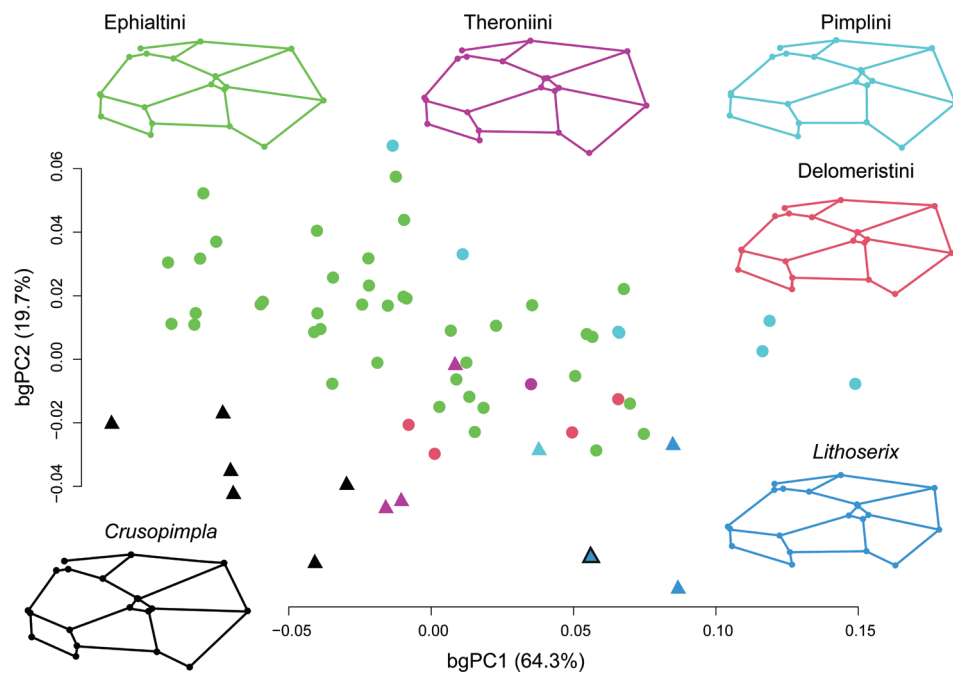


Figure 4. BgPCA of the fore wing in all specimens of the four pimpline tribes plus *Lithoserix* and *Crusopimpla*. The mean shape of each tribe/genus is shown in the respective colour. Triangles represent fossil species, whereas the blue triangle with black outline represents *Lithoserix oublieri* sp. nov.

Table 3. *P*-values of pairwise group differences in pimpline wings. Based on permutation testing of the bgPCA of fore wings and hind wings.

<i>Fore wing</i>	<i>Crusopimpla</i>	<i>Delomeristini</i>	<i>Ephialtini</i>	<i>Lithoserix</i>	<i>Pimplini</i>
<i>Delomeristini</i>	0.0118				
<i>Ephialtini</i>	0.0062	0.1425			
<i>Lithoserix</i>	0.0004	0.3245	0.0026		
<i>Pimplini</i>	0.0001	0.1025	0.0002	0.0932	
<i>Theroniini</i>	0.0870	0.4262	0.2375	0.1002	0.0563
<i>Hind wing</i>					
<i>Delomeristini</i>	0.1525				
<i>Ephialtini</i>	0.1970	0.7616			
<i>Lithoserix</i>	0.0358	0.409	0.0539		
<i>Pimplini</i>	0.0054	0.1545	0.0003	0.8323	
<i>Theroniini</i>	0.0505	0.3017	0.0469	0.3888	0.4231

All extant tribes do at least partially overlap in the first two bgPCA axes of the fore-wing variation. Additionally, while the fore wings shapes of Delomeristini and Theroniini do not differ much from most groups, the two larger extant tribes Pimplini and Ephialtini exhibit significant differences from each other (Table 3).

Pimpline group differences in hind wings

BgPC1 explains 79.3% of the overall shape variation, whereas bgPC2 explains 10.5% (Fig. 5). Here, no pimpline group appears to be completely isolated in the morphospace of the bgPCA. However, the two *Crusopimpla* species are located in the higher values of bgPC1 and lower values of bgPC2 and the opposite is observed in *Lithoserix*. Thus, those extinct genera do also distinctly differ from each other in their hind-wing

shape (Table 3). The mean shape of *Crusopimpla* is characterised by their rather long 1M vein and their nervellus. This nervellus is intercepted very low and veins 1Cu and cu-a are rather straight, while this interception is much more angled in the other groups. Those differences of the hind-wing shape are significantly different from Pimplini and Theroniini (Table 3) and the *Crusopimpla* hind-wing shape seems more similar to Ephialtini.

Lithoserix are located on the extreme lower value of bgPC1 and are characterised by a relatively long 1Rs compared to rs-m, a relative short 1M and their nervellus intercepted very high up. This is also observed in Pimplini and the mean shape of the two *Lithoserix* species is similar to the mean shape of Pimplini. There were also no significant differences observed in the pairwise comparison of the Procrustes distances of those two groups (Table 3). However, *Lithoserix* hind wings differ from species of Ephialtini (Table 3).

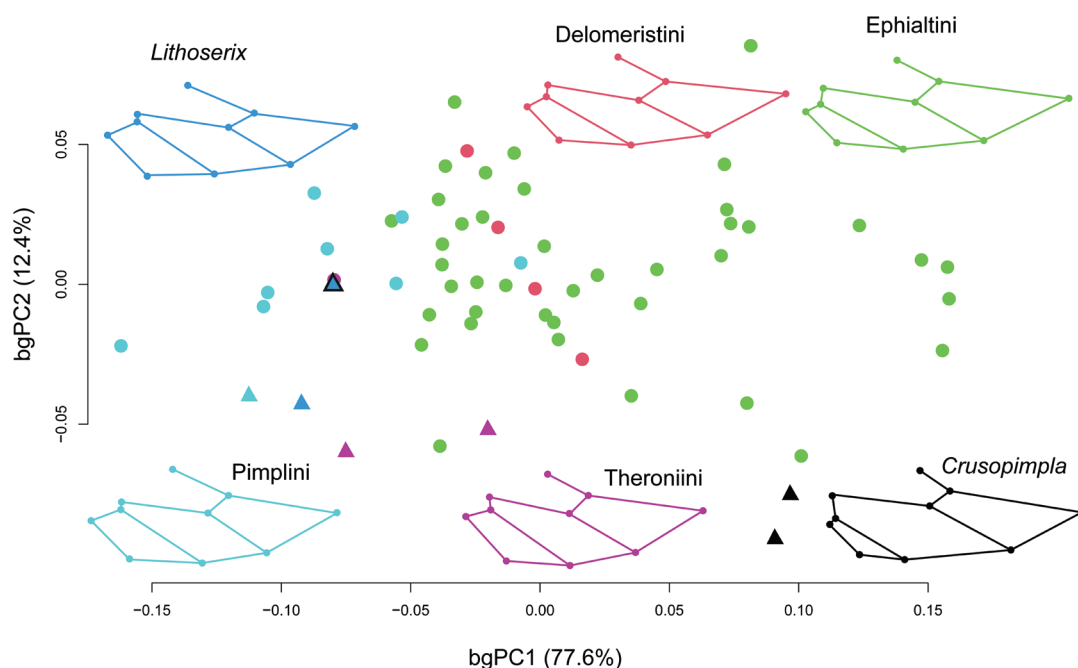


Figure 5. BgPCA of the hind wing in all specimens of the four pimpline tribes plus *Lithoserix* and *Crusopimpla*. The mean shape of each tribe/genus is shown in the respective colour. Triangles represent fossil species, whereas the blue triangle with black outline represents *Lithoserix oublieri* sp. nov.

Delomeristini occupies the morphospace mostly within Ephialtini and their hind wing mean shapes do appear similar, with just a slightly upwards shifted nervellus in Delomeristini. The Procrustes distances of their hind wings are not distinctly different (Table 3). The hind-wing shape of Theroniini is different from Ephialtini (Table 3), which appears to be caused by the higher cells M and 2Cu cells.

The CVA confirms the bgPCA results of fore and hind wings and can be found in Suppl. material 4.

Discussion

The new fossil species is the third species of the extinct pimpline genus *Lithoserix*, which lived from the late Eocene to the late Oligocene. In this study, I described the fossil species *Lithoserix oublieri* sp. nov. and evaluate the placement of *Lithoserix* within Pimplinae using of geometric morphometrics of the fore- and hind wings of extant and fossil pimpline taxa.

While the fore wing is useful to distinguish Pimplinae from other subfamilies (Viertler et al. 2022), it does not seem as straightforward between the tribes of extant Pimplinae, which all partially overlap in the bgPCA analyses. In the hind wings, however, some tribes exhibit a rather specific venation in the analysis. This is in agreement with previous suggestions that some groups can be distinguished through hind-wing venation: in *Crusopimpla*, the nervellus is broken below the middle (Klopfstein 2022) and, in Pimplini, it is broken way above the middle (Gauld et al. 2002). The fact that Pimplini splits in both bgPCA analyses from most Ephialtini,

Delomeristini and Theroniini might support the notion that it is not closely related to the remaining Pimplinae (Klopfstein et al. 2018).

Considering the species diversity of *Crusopimpla* (Klopfstein 2022; Viertler et al. 2022; Manukyan 2023) and its substantial dissimilarities of the wing venation from extant tribes and *Lithoserix* (see results), it is plausible that *Crusopimpla* may belong to an ancestral or basal pimpline tribe.

For *Lithoserix*, important characteristics, such as the clypeus shape or the tarsal claws, are missing to make a confident tribal assignment, as was already discussed with the two previously-described *Lithoserix* species (Spasojevic et al. 2022). However, based on the wing analyses conducted herein, *Lithoserix* should not be placed in the same tribe as *Crusopimpla* and also an affiliation to Ephialtini seems improbable. The wings of *Lithoserix* species are most similar to Pimplini. Both groups change shape similarly with increasing size and no significant shape differences were obtained in either fore- or hind wing. The hind wing with the nervellus intercepted high up, as found in *Lithoserix williamsi* and *Lithoserix oublieri* sp. nov., is often found in the tribe Pimplini, although it is less pronounced in *Lithoserix antiquus*. An argument against Pimplini is the ovipositor length of *Lithoserix*, which is not completely preserved in this fossil, but very long in *Lithoserix williamsi* and *L. antiquus*. This is rather rare in Pimplini, but frequent in Ephialtini (Gauld et al. 2002). However, because Pimplinae probably originated in the Cretaceous (Spasojevic et al. 2021), it would not be surprising to find *Lithoserix* belonging to a stem-lineage within Pimplini or even an extinct tribe, possibly closely-related Pimplini.

Conclusion

To confidently place *Lithoserix* in Pimplini or to propose a new tribe, a more robust collection of fossils and comprehensive morphological evidence is imperative. The classification of *Lithoserix* presents challenges, as it appears to be situated closest to Pimplini, when considering wing venation, but not according to ovipositor length. To gain information on the evolutionary path of Pimplinae or Darwin wasps, in general, more fossils need to be evaluated.

However, this might be a difficult task. There is not only a lack of researchers studying the astonishing diversity of extant Darwin wasp, but even more so of people working with their fossil taxa. It is probably not rare that undescribed Darwin wasp fossils are labelled “wasp” or “Hymenoptera” in natural history collections and we can only speculate how many fossils in this group are currently overlooked.

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Supplementary material 1

Taxon list

Authors: Alexandra Viertler

Data type: xlsx

Explanation note: This table shows all included extant and fossil taxa that were used in the geometric morphometric analyses of the fore- and hind wings.

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Link: <https://doi.org/10.3897/fr.27.116373.suppl1>

Supplementary material 2

TPS dataset of fore wings

Authors: Alexandra Viertler

Data type: tps

Explanation note: This dataset includes 21 fixed landmarks, of which the first landmark was excluded. It also contains two curves: The first curve (eight semi-landmarks) was placed between the landmarks delimiting vein 2m-cu and the second curve (ten semi-landmarks) was placed between the landmarks delimiting vein 1m-cu & 2Rs+M. Both curves were ignored in this analysis

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Link: <https://doi.org/10.3897/fr.27.116373.suppl2>

Supplementary material 3

TPS dataset of hind wings

Authors: Alexandra Viertler

Data type: tps

Explanation note: This dataset includes 11 fixed landmarks, of which the first landmark was excluded for this analysis because it represented the start of vein Sc+R on the hind-wing base, which is often not visible in fossil taxa.

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Link: <https://doi.org/10.3897/fr.27.116373.suppl3>

Supplementary material 4

Canonical variation analysis (CVA) of fore- and hind wings

Authors: Alexandra Viertler

Data type: tif

Explanation note: This figure shows the CVA of **A.** fore wings and **B.** hind wings of four pimpline tribes and two extinct genera. Both plots show the first two axes of the respective CVA analysis and the extreme shape change along the labelled axes. Triangles represent fossil species, whereas the blue triangle with black outline represents *Lithoserix oublieri* sp. nov. **A.** In the fore wing, CV1 (50.5% explained variance) separates Ephialtini, Pimplini and Delomeristiini from Theronini and the two extinct genera, *Crusopimpla* and *Lithoserix*. The shape change mostly effects the angle of the distal part of the fore wing (LM 3, 19, 20).

CV2 (22.4% explained variance) separates Pimplini to one extreme, *Crusopimpla* to the other extreme from the other groups Ephialtini, Theronini and *Lithoserix*. Here the shape change includes broadening (2R1, 2M) or shortening (1M+1R1, 2Cu) of various cells in the lower values (red outline). **B.** In the hind wing, CV1 (47.4% explained variance) puts *Lithoserix* and many Pimplini in the higher extremes, with the nervellus intercepted above the middle. CV2 (20.3% explained variance) splits mostly *Crusopimpla*, with the nervellus intercepted below the middle, from the other groups. Delomeristiini are situated within Ephialtini.

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Link: <https://doi.org/10.3897/fr.27.116373.suppl4>

Supplementary material 5

Results of Canonical variation analysis (CVA) of fore and hind wings

Authors: Alexandra Viertler

Data type: docx

Explanation note: Short results section of the CVA analyses of fore and hind wings in Pimplinae.

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