Response to Comments of Anonymous Referee #1

Dear Prof. Dr. Alessio Rovere,

Thank you very much for handling our manuscript essd-2022-23 entitled "*Artemisia* pollen dataset for exploring the potential ecological indicators in deep time" and moving it forward. Right now, we have completed our revisions following the comments of anonymous referee #1. We hope this new version is suitable for publication in your journal.

Attached below are the items we respond to the comments posted by referee #1 and structure the response followed by the sequence: (1) reviewer comment, (2) author's response, and (3) changes to manuscript. Please let us know if further changes are required.

Yours Sincerely,

Yu-Fei Wang and co-authors

Responses to referee #1

Reviewer comment 1: I thank the authors for addressing my comments in detail, and generating additional data to add to their dataset. I just have a two minor suggestions for revision:

Author's response: We appreciate the time and effort you spent on reviewing. We have revised the manuscript according to your comments. Please see the following point-by-point responses.

Reviewer comment 2: Line 24: suggest changing to '9360 pollen morphological trait measurements' - the traits are usually considered to be the characters that you are measuring, rather than the individual measurements.

Author's response: Done.

Changes to manuscript: We changed "9360 statistical pollen morphological traits" to "9360 pollen morphological trait measurements" in Abstract, Table 3 and the description of dataset.

Reviewer comment 3: Intraspecific variability (reviewer comment 3 in the authors' response to my review): there are additional measurements and analyses here but they are only in the review response, not the paper. It would be much more useful to readers to work these into the manuscript, including Figure R2, and to include the additional measurements in the supplementary material (this could be a separate sheet in the 'Statistical pollen morphological traits' Excel file).

Author's response: Thank you for your helpful suggestions and improvements on the manuscript!

Changes to manuscript: 1. We added this part into the revised manuscript as an independent section 4.2 Testing the pollen intraspecific variability within *Artemisia* (lines 361-387).

"4.2 Testing the pollen intraspecific variability within *Artemisia*

Evidence shows that the pollen morphology in *Artemisia* is highly uniform under LM without discrimination (Wodehouse, 1926; Sing and Joshi, 1969; Ling, 1982; Chen, 1987; Wang et al., 1995), which might suggest that statistical analyses of the intraspecific morphological variability of pollen under the LM are limited or meaningless. Right now, the SEM technique has made it possible to subdivide *Artemisia* pollen into different types using pollen exine ultrastructure characters (Chen, 1987; Chen and Zhang, 1991; Sun and Xu, 1997; Jiang et al., 2005; Ghahraman et al., 2007; Shan et al., 2007; Hayat et al., 2009; Hayat et al., 2010; Hussain et al., 2019).

In order to test the intraspecific variability of pollen exine ultrastructure traits, we selected one species respectively from the three pollen types corresponding to the three morphological clades of *Artemisia* pollen, i.e. *Artemisia vulgaris* (SWS type), *Artemisia annua* (LNS type), and *Artemisia maritima* (SG type), and sampled five specimens of each species (Table B2). Six pollen traits, i.e. D, H, D/H, Gs, Ss, and Gs/Ss, were counted and analysed under SEM to test for intraspecific variability of pollen exine ultrastructure traits.

The test showed that it was feasible to use stable D/H and Gs/Ss for pollen type classification of *Artemisia* because 1) D/H and Gs/Ss were stable within species (Figure 20, Table 2) for the pollen classification; 2) D, H, Gs, and Ss were variable as size values, e.g. these four traits were significantly different within species in both *A. vulgaris* and *A. annua*, while D, H, and Ss were significantly different within species in *A. maritima* (Figure 20, Table 2). There was evidence showing that size values such as pollen exine ultrastructure size were often variable within species due to their genetic divergence, various habitats, and different experimental treatments (Mo et al., 1997; Zhao and Yao, 1999; Zhang and Qian, 2011)."

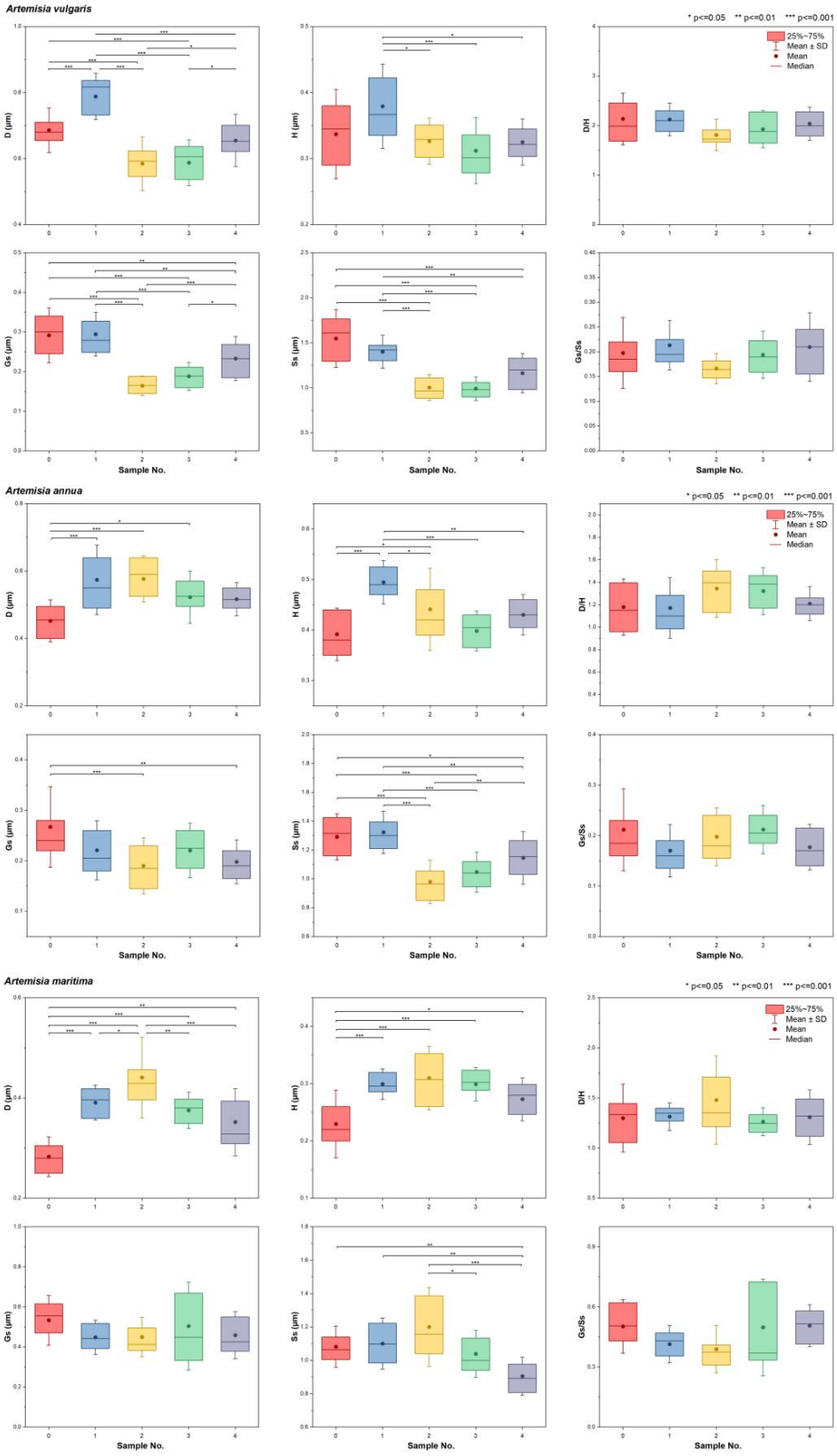

Figure 20. Boxplots of intraspecific pollen exine ultrastructure characters from three species of *Artemisia*, showing the variations (M ± SD) in six pollen characters (D: diameter of spinule base; H: spinule height; Gs: granule spacing; Ss: spinule spacing; Ps: perforation spacing). Asterisks indicate statistically significant differences (*p <= 0.05, **p <= 0.01, ***p < 0.001).

Table 2. The results of ANOVA for intraspecific variability in pollen exine ultrastructure characters among three representative species.

Pollen exine ultrastructure characters	SWS type	LNS type	SG type
	Artemisia vulgaris	***Artemisia annua***	***Artemisia maritima***
D (μm)	significant	significant	significant
H (μm)	significant	significant	significant
D/H	non-significant	non-significant	non-significant
Gs (μm)	significant	significant	significant
Ss (μm)	significant	significant	significant
Gs/Ss	non-significant	non-significant	non-significant

2. We added Table B2 in section Appendix B listing the voucher specimens (lines 746-748).

Table B2. List of the voucher specimens in PE Herbarium, Institute of Botany, Chinese Academy of Sciences. Sample No. 0 was the specimen for cluster analysis. Sample Nos.1-4 were used in testing intraspecific variability in pollen exine ultrastructure characters among three representative species.

Sample No.	SWS type		LNS type		SG type	
	Artemisia vulgaris		***Artemisia annua***		***Artemisia maritima***	
	Specimen barcodes	**Coll. No.**	**Specimen barcodes**	**Coll. No.**	**Specimen No.**	**Coll. No.**
0	PE 01669703	P.Frost-Olsen 1833	PE 01197344	Wen-Hong Jin-Tian, Kai-Yong Lang, Ge Yang 328	No. 1338063	s.n.
1	PE 00532417	75-1521	PE 420433	Xue-Zhong Liang 328	No. 209452	G. Belloteau 1912.9.25
2	PE 00492025	K.M.Liou L.6601	PE 420647	T.N.Liou & C.Wang 731	No. 209446	s.n.
3	PE 00492038	P.C.Hoch, Jia-Rui Chen 86077	PE 420660	Da-Shun Wang 856	s.n.	Hanelt Schultze-Motel 446
4	PE 00492029	Hong-Bin Cui, You-Run Lin, Zhen-Dai Xia 80-290	PE 420664	Lei,C.I. 858	s.n.	O. Nordstedt 1901-10-9

3. We revised Table 3 in section 5 Data availability including the additional measurements of the intraspecific variability test (line 434).

Table 3. *Artemisia* pollen datasets in this study.

Data type	Data format	Data acquisition	Data accessibility
The phylogenetic framework of *Artemisia* pollen sampling.	.png	Literature survey (modified from Malik et al., 2017).	This article
A voucher specimen list of 36 representative species.	.doc	Pollen samples were obtained from PE herbarium at the Institute of Botany, Chinese Academy of Sciences.	
12 illustrations of pollen grains and the habitats of their source plants.	.png	Habitat photos from online sources (Appendix Table A).	
4018 original pollen photographs (3205 under LM, 813 under SEM).	.jpg	Pollen samples were acetolyzed by the standard method and fixed in glycerine jelly. The pollen grains were photographed under LM and SEM using standard procedures.	Zenodo (https://doi.org/10.5281/zenodo.6900308; Lu et al., 2022)
9360 pollen morphological trait measurements of 36 representative species.	.xlsx	Statistical data of pollen morphological traits were measured by standard methods.	
1800 pollen morphological trait measurements for testing the pollen intraspecific variability within *Artemisia*.	.xlsx	Statistical data of pollen morphological traits were measured by standard methods.	
30858 source plant occurrence information, and corresponding environmental factors including altitude and 19 climate parameters.	.xlsx	Their source plant distribution coordinates were obtained from GBIF (https://doi.org/10.15468/dl.596xd9). The corresponding environmental factors of these coordinates were obtained from WorldClim (https://www.worldclim.org/) with a spatial resolution of 30 seconds between 1970-2000.	

4. These additional measurements of intraspecific variability have been added to the “Statistical pollen morphological traits” Excel file as a new sheet in datasets available at Zenodo (https://doi.org/10.5281/zenodo.6900308).