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Aspects of the analysis of cell imagery: from shape to understanding

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Appendix A

Supplementary Materials in Chapter 2

Supplementary Table S1

Locations of all Urticaceae specimens and number of images. NL = the Netherlands, SP = Spain and PO = Portugal. *collected in 2018 and 2019, deposited in the Naturalis Biodiversity Center herbarium.

Species (n=total images)	**Geographical origin**	**Collection date**	**No. of images used**	**Deposition number**
Parietaria judaica L. (n = 1670)	Montejaque (SP)	17/10/2011	54	WAG.1186948
	Leiden, Stationsweg (NL)	19/11/2019	168	L.3993376*
	Huizen (NL)	20/09/2014	174	L.4303913
	Leiden, Robijnstraat (NL)	23/07/2012	139	L.2071680
	Den Haag (NL)	05/10/2018	392	L.3993377*
	Leiden, Paterstraatje	09/10/2018	250	L.3993378*
	Sassenplaat (NL)	03/07/2013	233	L.4304093
	Rotterdam, Hartelkanaal (NL)	27/09/2014	260	L.4304136
Parietaria officinalis L. (n = 1359)	Middelburg (NL)	26/06/2014	234	L.3974371
	Haarlem (NL)	13/07/2013	191	L.2073373
	Wageningse Polder (NL)	19/07/2012	64	WAG.1186992
	Leiden (NL)	07/2012	369	L.3963901
	Den Haag, Escamplaan (NL)	12/10/2018	383	L.3993379*
	Den Haag, Bosjes van Poot (NL)	01/08/2012	248	L.2071818
Urtica dioica L. (n = 1055)	Leiden, Hogeschool 1 (NL)	06/11/2019	316	L.3993380*
	Leiden, Hogeschool 2 (NL)	07/11/2019	299	L.3993381*
	Den Haag (NL)	17/11/2019	182	L.3993382*

	Leiden, Sandifortdreef (NL)	15/11/2019	191	L.3993383*
	Arnhem (NL)	29/05/2001	67	WAG.1188104
Urtica membranacea Poir. ex Savigny (n = 1118)	Amsterdam (NL)	11/2018	521	L.3993384*
	Overloon (NL)	17/06/2014	135	L.3959964
	Cape st. Vincent (PO)	03/1995	87	L.1629741
	Leiden, Sandifortdreef (NL)	15/11/2019	191	L.3993383*
	Den Haag (NL)	06/03/2019	375	L.3993385*
Urtica urens L. (n = 1270)	Leiden (NL)	01/11/2019	128	L.3993386*
	Castilla-la-Mancha (SP)	27/05/2016	165	WAG.1962413
	Zandvoort (NL)	05/08/2012	201	L.2071917
	Meijendel (NL)	12/08/2011	140	L.2074446
	Zwolle (NL)	29/04/2005	134	L.4271105
	Wassenaar (NL)	15/09/2002	219	L.4233917
	Den Haag (NL)	13/03/2020	283	L.3993387*

Supplementary Table S2

Probability scores for Urticaceae pollen grains scanned from aerobiological samples using the pre-trained VGG16 model with 5-fold cross-validation. *U. mem* = *Urtica membranacea*

Lleida (16-06-2019), n=63

Image No.	**Probability *Parietaria***	**Probability *Urtica***	**Probability *U.mem***	**Final ID Threshold 0.6**	**Final ID Threshold 0.7**
1	0.95	0.05	0.00	*Parietaria*	*Parietaria*
2	0.98	0.02	0.00	*Parietaria*	*Parietaria*
3	0.29	0.70	0.01	*Urtica*	*Urtica*
4	0.98	0.02	0.00	*Parietaria*	*Parietaria*
5	0.24	0.76	0.00	*Urtica*	*Urtica*
6	1.00	0.00	0.00	*Parietaria*	*Parietaria*
7	0.94	0.06	0.00	*Parietaria*	*Parietaria*
8	0.99	0.01	0.00	*Parietaria*	*Parietaria*
9	0.12	0.88	0.00	*Urtica*	*Urtica*
10	0.99	0.01	0.00	*Parietaria*	*Parietaria*

11	0.99	0.01	0.00	*Parietaria*	*Parietaria*
12	0.96	0.04	0.00	*Parietaria*	*Parietaria*
13	1.00	0.00	0.00	*Parietaria*	*Parietaria*
14	0.96	0.04	0.00	*Parietaria*	*Parietaria*
15	1.00	0.00	0.00	*Parietaria*	*Parietaria*
16	0.90	0.09	0.01	*Parietaria*	*Parietaria*
17	0.90	0.01	0.09	*Parietaria*	*Parietaria*
18	0.73	0.16	0.10	*Parietaria*	*Parietaria*
19	0.95	0.04	0.00	*Parietaria*	*Parietaria*
20	0.98	0.00	0.02	*Parietaria*	*Parietaria*
21	0.16	0.83	0.00	*Urtica*	*Urtica*
22	0.67	0.31	0.02	*Parietaria*	unknown
23	0.02	0.98	0.00	*Urtica*	*Urtica*
24	0.95	0.04	0.00	*Parietaria*	*Parietaria*
25	0.99	0.01	0.00	*Parietaria*	*Parietaria*
26	0.99	0.01	0.00	*Parietaria*	*Parietaria*
27	0.95	0.05	0.00	*Parietaria*	*Parietaria*
28	0.02	0.98	0.00	*Urtica*	*Urtica*
29	0.34	0.66	0.00	*Urtica*	unknown
30	1.00	0.00	0.00	*Parietaria*	*Parietaria*
31	0.98	0.02	0.00	*Parietaria*	*Parietaria*
32	1.00	0.00	0.00	*Parietaria*	*Parietaria*
33	0.99	0.01	0.00	*Parietaria*	*Parietaria*
34	0.92	0.02	0.06	*Parietaria*	*Parietaria*
35	0.57	0.41	0.02	unknown	unknown
36	1.00	0.00	0.00	*Parietaria*	*Parietaria*
37	0.87	0.13	0.00	*Parietaria*	*Parietaria*
38	0.99	0.00	0.00	*Parietaria*	*Parietaria*
39	0.97	0.03	0.00	*Parietaria*	*Parietaria*
40	0.58	0.41	0.01	unknown	unknown
41	0.98	0.02	0.00	*Parietaria*	*Parietaria*
42	0.70	0.29	0.00	*Parietaria*	*Parietaria*
43	0.84	0.16	0.00	*Parietaria*	*Parietaria*
44	0.97	0.02	0.01	*Parietaria*	*Parietaria*
45	0.83	0.17	0.00	*Parietaria*	*Parietaria*
46	0.99	0.00	0.00	*Parietaria*	*Parietaria*

47	1.00	0.00	0.00	*Parietaria*	*Parietaria*
48	0.99	0.00	0.00	*Parietaria*	*Parietaria*
49	0.96	0.04	0.01	*Parietaria*	*Parietaria*
50	0.00	1.00	0.00	*Urtica*	*Urtica*
51	0.99	0.01	0.00	*Parietaria*	*Parietaria*
52	0.99	0.00	0.01	*Parietaria*	*Parietaria*
53	0.91	0.04	0.05	*Parietaria*	*Parietaria*
54	0.95	0.04	0.00	*Parietaria*	*Parietaria*
55	0.90	0.10	0.00	*Parietaria*	*Parietaria*
56	0.95	0.05	0.00	*Parietaria*	*Parietaria*
57	0.99	0.01	0.00	*Parietaria*	*Parietaria*
58	1.00	0.00	0.00	*Parietaria*	*Parietaria*
59	0.99	0.01	0.00	*Parietaria*	*Parietaria*
60	0.17	0.82	0.00	*Urtica*	*Urtica*
61	0.41	0.56	0.02	unknown	unknown
62	0.98	0.02	0.00	*Parietaria*	*Parietaria*
63	0.76	0.21	0.03	*Parietaria*	*Parietaria*

Vielha (09-08-2019), n=26

Image No.	**Probability *Parietaria***	**Probability *Urtica***	**Probability *U.mem***	**Final ID Threshold 0.6**	**Final ID Threshold 0.7**
1	0.03	0.97	0.00	*Urtica*	*Urtica*
2	0.07	0.86	0.07	*Urtica*	*Urtica*
3	0.10	0.90	0.00	*Urtica*	*Urtica*
4	0.02	0.98	0.00	*Urtica*	*Urtica*
5	0.09	0.91	0.00	*Urtica*	*Urtica*
6	0.26	0.74	0.00	*Urtica*	*Urtica*
7	0.00	1.00	0.00	*Urtica*	*Urtica*
8	0.41	0.04	0.55	unknown	unknown
9	0.61	0.39	0.01	*Parietaria*	unknown
10	0.81	0.10	0.09	*Parietaria*	*Parietaria*
11	0.02	0.98	0.00	*Urtica*	*Urtica*
12	0.01	0.99	0.00	*Urtica*	*Urtica*
13	0.49	0.13	0.38	unknown	unknown

14	0.00	1.00	0.00	*Urtica*	*Urtica*
15	0.14	0.84	0.02	*Urtica*	*Urtica*
16	0.63	0.10	0.27	*Parietaria*	unknown
17	0.12	0.88	0.00	*Urtica*	*Urtica*
18	0.09	0.90	0.10	*Urtica*	*Urtica*
19	0.24	0.76	0.00	*Urtica*	*Urtica*
20	0.04	0.96	0.00	*Urtica*	*Urtica*
21	0.85	0.12	0.03	*Parietaria*	*Parietaria*
22	0.80	0.14	0.07	*Parietaria*	*Parietaria*
23	0.00	1.00	0.00	*Urtica*	*Urtica*
24	0.17	0.83	0.00	*Urtica*	*Urtica*
25	0.02	0.98	0.00	*Urtica*	*Urtica*
26	0.57	0.43	0.00	unknown	unknown

Leiden (23-08-2019), n=112

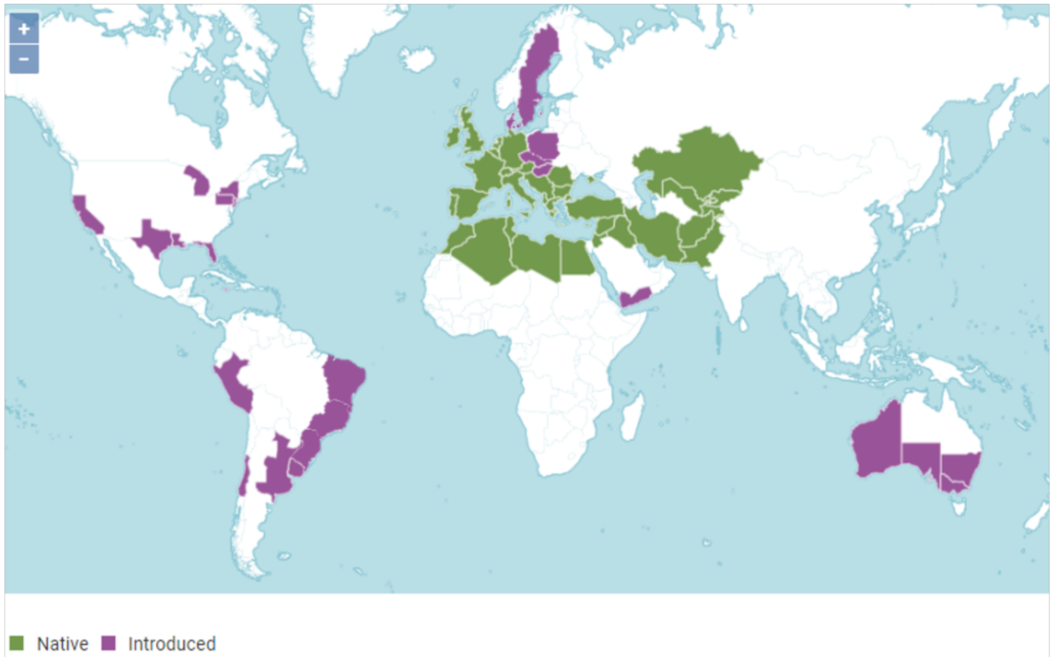
Image No.	**Probability *Parietaria***	**Probability *Urtica***	**Probability *U.mem***	**Final ID Threshold 0.6**	**Final ID Threshold 0.7**
1	0.04	0.96	0.00	*Urtica*	*Urtica*
2	0.01	0.99	0.00	*Urtica*	*Urtica*
3	0.07	0.93	0.00	*Urtica*	*Urtica*
4	0.16	0.83	0.00	*Urtica*	*Urtica*
5	0.19	0.81	0.00	*Urtica*	*Urtica*
6	0.02	0.98	0.00	*Urtica*	*Urtica*
7	0.00	1.00	0.00	*Urtica*	*Urtica*
8	0.28	0.72	0.00	*Urtica*	*Urtica*
9	0.11	0.89	0.00	*Urtica*	*Urtica*
10	0.34	0.66	0.00	*Urtica*	unknown
11	0.04	0.96	0.00	*Urtica*	*Urtica*
12	0.18	0.81	0.00	*Urtica*	*Urtica*
13	0.00	1.00	0.00	*Urtica*	*Urtica*
14	0.47	0.53	0.00	unknown	unknown
15	0.11	0.89	0.00	*Urtica*	*Urtica*
16	0.01	0.99	0.00	*Urtica*	*Urtica*
17	0.20	0.80	0.00	*Urtica*	*Urtica*

18	0.00	1.00	0.00	*Urtica*	*Urtica*
19	0.00	1.00	0.00	*Urtica*	*Urtica*
20	0.01	0.99	0.00	*Urtica*	*Urtica*
21	0.75	0.25	0.00	*Parietaria*	*Parietaria*
22	0.00	1.00	0.00	*Urtica*	*Urtica*
23	0.03	0.97	0.00	*Urtica*	*Urtica*
24	0.01	0.99	0.00	*Urtica*	*Urtica*
25	0.69	0.31	0.00	*Parietaria*	unknown
26	0.11	0.89	0.00	*Urtica*	*Urtica*
27	0.12	0.88	0.00	*Urtica*	*Urtica*
28	0.17	0.83	0.00	*Urtica*	*Urtica*
29	0.09	0.91	0.00	*Urtica*	*Urtica*
30	0.00	1.00	0.00	*Urtica*	*Urtica*
31	0.48	0.52	0.00	unknown	unknown
32	0.24	0.76	0.00	*Urtica*	*Urtica*
33	0.06	0.94	0.00	*Urtica*	*Urtica*
34	0.29	0.71	0.00	*Urtica*	*Urtica*
35	0.14	0.86	0.00	*Urtica*	*Urtica*
36	0.38	0.62	0.00	*Urtica*	unknown
37	0.06	0.94	0.00	*Urtica*	*Urtica*
38	0.55	0.45	0.00	unknown	unknown
39	0.01	0.99	0.00	*Urtica*	*Urtica*
40	0.00	1.00	0.00	*Urtica*	*Urtica*
41	0.00	1.00	0.00	*Urtica*	*Urtica*
42	0.02	0.98	0.00	*Urtica*	*Urtica*
43	0.03	0.97	0.00	*Urtica*	*Urtica*
44	0.21	0.79	0.00	*Urtica*	*Urtica*
45	0.02	0.98	0.00	*Urtica*	*Urtica*
46	0.00	1.00	0.00	*Urtica*	*Urtica*
47	0.01	0.99	0.00	*Urtica*	*Urtica*
48	0.79	0.20	0.07	*Parietaria*	*Parietaria*
49	0.54	0.46	0.00	unknown	unknown
50	0.01	0.99	0.00	*Urtica*	*Urtica*
51	0.00	1.00	0.00	*Urtica*	*Urtica*
52	0.01	0.99	0.00	*Urtica*	*Urtica*
53	0.01	0.99	0.00	*Urtica*	*Urtica*

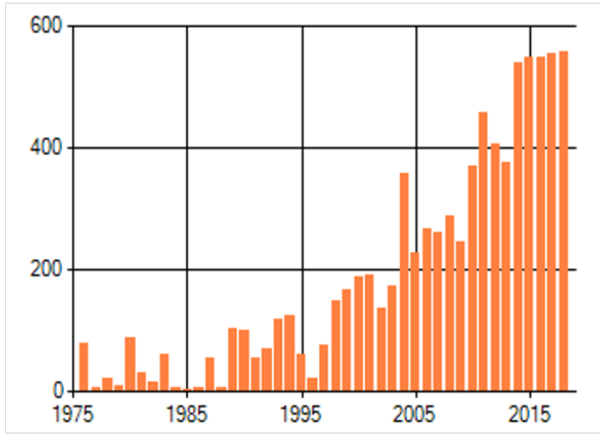
54	0.00	1.00	0.00	*Urtica*	*Urtica*
55	0.00	1.00	0.00	*Urtica*	*Urtica*
56	0.00	1.00	0.00	*Urtica*	*Urtica*
57	0.02	0.98	0.00	*Urtica*	*Urtica*
58	0.00	1.00	0.00	*Urtica*	*Urtica*
59	0.54	0.46	0.00	unknown	unknown
60	0.45	0.55	0.00	unknown	unknown
61	0.09	0.91	0.00	*Urtica*	*Urtica*
62	0.00	1.00	0.00	*Urtica*	*Urtica*
63	0.00	1.00	0.00	*Urtica*	*Urtica*
64	0.00	1.00	0.00	*Urtica*	*Urtica*
65	0.06	0.94	0.00	*Urtica*	*Urtica*
66	0.05	0.95	0.00	*Urtica*	*Urtica*
67	0.01	0.99	0.00	*Urtica*	*Urtica*
68	0.23	0.77	0.00	*Urtica*	*Urtica*
69	0.21	0.79	0.00	*Urtica*	*Urtica*
70	0.72	0.28	0.00	*Parietaria*	*Parietaria*
71	0.49	0.51	0.00	unknown	unknown
72	0.06	0.94	0.00	*Urtica*	*Urtica*
73	0.33	0.67	0.00	*Urtica*	unknown
74	0.00	1.00	0.00	*Urtica*	*Urtica*
75	0.28	0.72	0.00	*Urtica*	*Urtica*
76	0.00	1.00	0.00	*Urtica*	*Urtica*
77	0.03	0.97	0.00	*Urtica*	*Urtica*
78	0.05	0.95	0.00	*Urtica*	*Urtica*
79	0.21	0.79	0.00	*Urtica*	*Urtica*
80	0.00	1.00	0.00	*Urtica*	*Urtica*
81	0.00	1.00	0.00	*Urtica*	*Urtica*
82	0.03	0.97	0.00	*Urtica*	*Urtica*
83	0.02	0.98	0.00	*Urtica*	*Urtica*
84	0.12	0.88	0.00	*Urtica*	*Urtica*
85	0.17	0.83	0.00	*Urtica*	*Urtica*
86	0.01	0.99	0.00	*Urtica*	*Urtica*
87	0.90	0.10	0.00	*Parietaria*	*Parietaria*
88	0.11	0.89	0.00	*Urtica*	*Urtica*
89	0.02	0.98	0.00	*Urtica*	*Urtica*

90	0.00	1.00	0.00	*Urtica*	*Urtica*
91	0.29	0.71	0.00	*Urtica*	*Urtica*
92	0.11	0.89	0.00	*Urtica*	*Urtica*
93	0.12	0.88	0.00	*Urtica*	*Urtica*
94	0.01	0.99	0.00	*Urtica*	*Urtica*
95	0.01	0.99	0.00	*Urtica*	*Urtica*
96	0.00	1.00	0.00	*Urtica*	*Urtica*
97	0.00	1.00	0.00	*Urtica*	*Urtica*
98	0.02	0.98	0.00	*Urtica*	*Urtica*
99	0.00	1.00	0.00	*Urtica*	*Urtica*
100	0.21	0.79	0.00	*Urtica*	*Urtica*
101	0.55	0.45	0.00	unknown	unknown
102	0.00	1.00	0.00	*Urtica*	*Urtica*
103	0.48	0.52	0.00	unknown	unknown
104	0.00	1.00	0.00	*Urtica*	*Urtica*
105	0.00	1.00	0.00	*Urtica*	*Urtica*
106	0.57	0.43	0.00	unknown	unknown
107	0.11	0.89	0.00	*Urtica*	*Urtica*
108	0.23	0.77	0.00	*Urtica*	*Urtica*
109	0.26	0.74	0.00	*Urtica*	*Urtica*
110	0.12	0.88	0.00	*Urtica*	*Urtica*
111	0.58	0.42	0.00	unknown	unknown
112	0.00	1.00	0.00	*Urtica*	*Urtica*

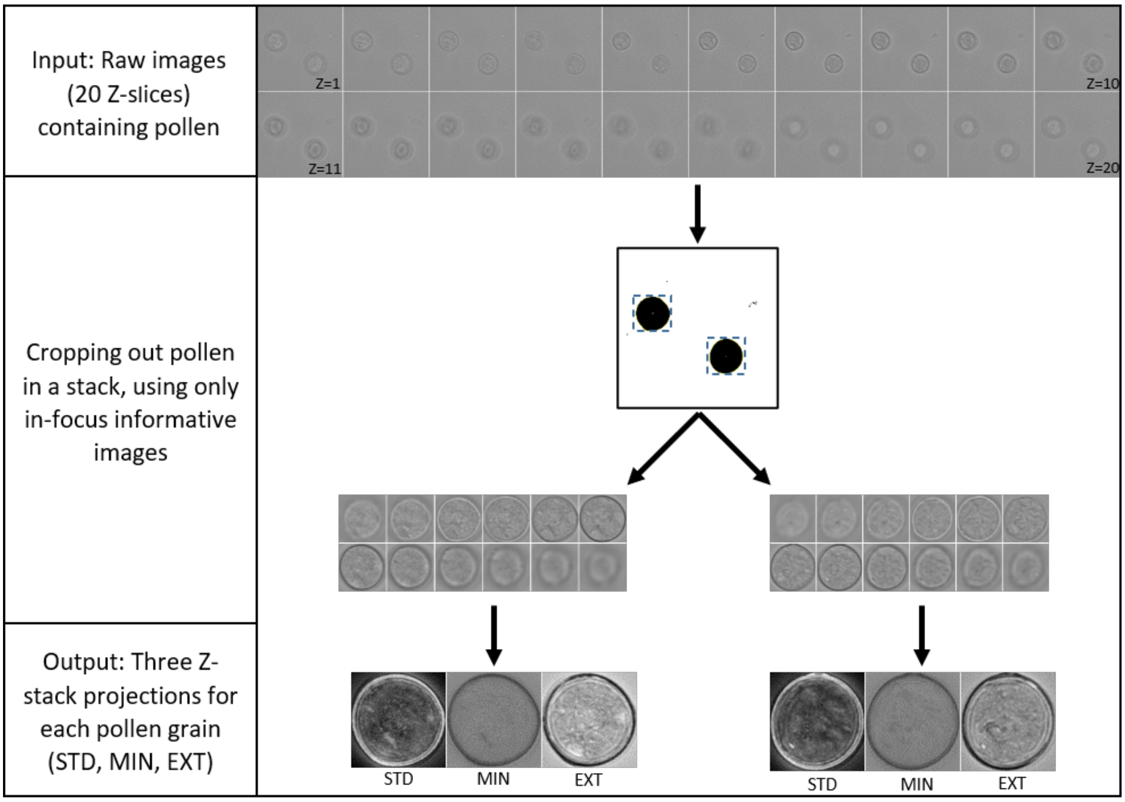
(a)



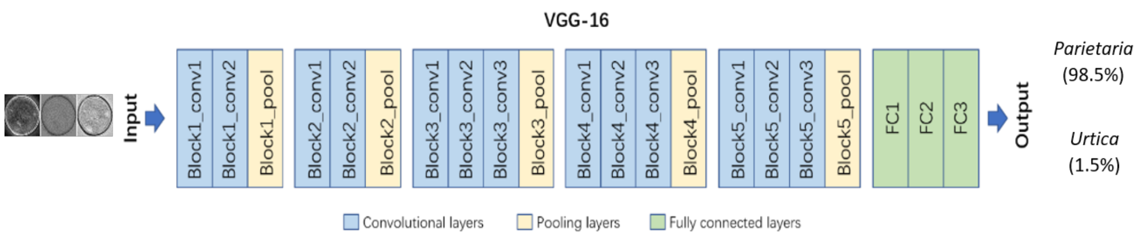
(b)



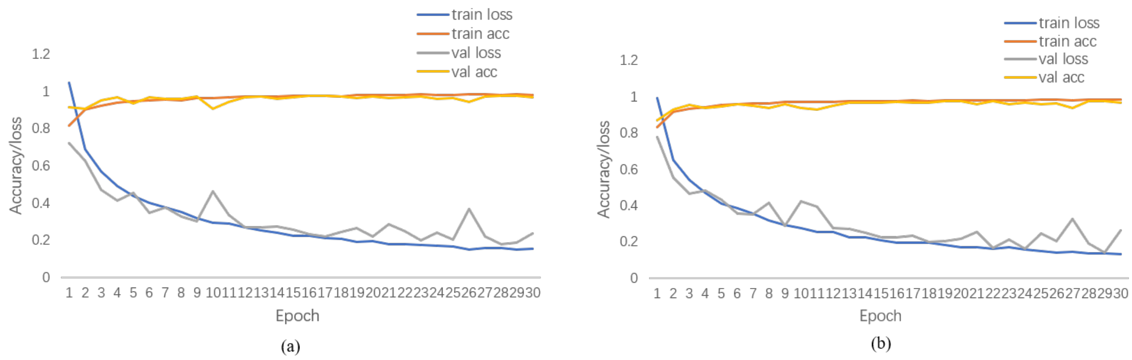
Supplementary Fig. S1. (a) Global native (green) and introduced (purple) distribution of *Parietaria judaica* and *P. officinalis* (POWO (2019). "Plants of the World Online. Map taken from the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/ Retrieved 05 October 2020"). (b) Trend in Pellitory of the wall (*Parietaria judaica*) plant sightings per square kilometre in the Netherlands over the past 45 years. Index number = 100 for 1990 © NEM (CBS FLORON) 2019.



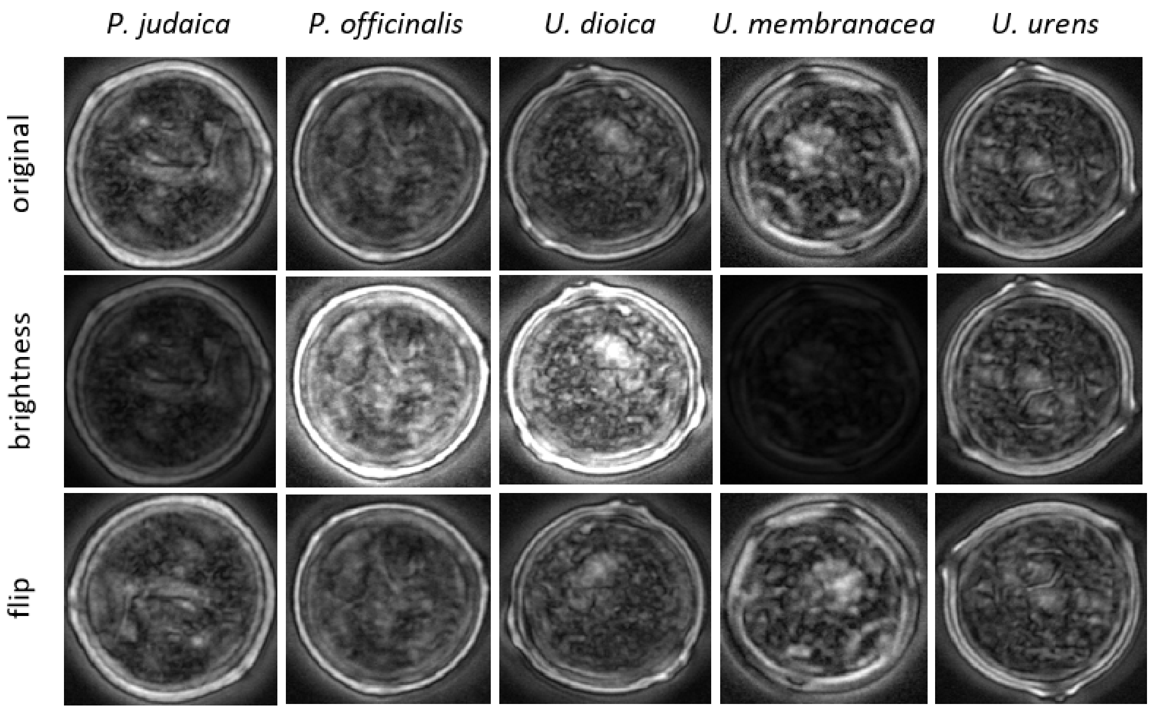
Supplementary Fig. S2. Pollen image acquisition and processing workflow carried out with in-house designed Pollen_Projector script. Once raw images are obtained at 20 different focal levels ('Z-slices'), subsequent steps involve cropping of whole individual pollen grains and producing three different projections from the Z-stacks. Abbreviations of projections: STD = Standard Deviation, MIN = Minimum Intensity and EXT = Extended Focus.



Supplementary Fig. S3. Schematic overview of the structure of VGG-16 with an example of three-channel input image of a *Parietaria judaica* pollen grain (known label) and the output generated, where it confidently identifies the images as *Parietaria* (98% probability). Adapted from Simonyan et al., (2014)[1]



Supplementary Fig. S4. Figures showing the accuracy/loss plots for the VGG16 model with 5- and 10-fold cross-validation.



Supplementary Fig. S5. Examples of data augmentation on the Standard Deviation Projection (STD) of selected pollen grains of all Urticaceae pollen species used in this study.

Appendix B

Supplementary Materials in Chapter 3

Supplementary Table S1 The classification performance of deep learning models on 5-species dataset. Standard deviation, training each model three times, is given in brackets.

	Precision	**Recall**	**F1 score**
VGG16	0.978(±0.001)	0.977(±0.001)	0.977(±0.001)
VGG19	0.974(±0.002)	0.973(±0.002)	0.973(±0.002)
ResNet50	0.980(±0.001)	0.979(±0.001)	0.979(±0.001)
MobileNet V1	0.958(±0.003)	0.957(±0.003)	0.957(±0.003)
MobileNet V2	0.970(±0.006)	0.970(±0.006)	0.970(±0.006)

Supplementary Table S2 The details of the dataset used in our study.

Classes	**Species**	**The number of images**	**The number of individual plants**
Parietaria	*Parietaria Judaica*	1670	8
	Parietaria officinalis	1359	6
Urtica	*Urtica dioica*	1055	5
	Urtica urens	1270	7
Urtica membranacea	*Urtica membranacea*	1118	4
Total images/individual plants were analyzed		6472	30

Supplementary Table S3 The final size of feature vector after feature selection/reduction.

Classifier	**Feature selection/reduction**	**The final size after feature selection/reduction**
SVM	PCA (0.8)	179×1
RF	SelectFromModel (mean)	2064×1
MLP	PCA (0.85)	337×1
Adaboost	Mutual information (2000)	2000×1

Supplementary Table S4 The number of images of predicted results for Urticaceae pollen grains scanned from aerobiological samples using the VGG16 and ResNet50 model with 5-fold cross-validation. *U. mem* = *Urtica membranacea*.

Location	**Leiden, the Netherlands**				**Lleida, Spain**			
Class labels	Label 1: *Parietaria*	Label 2: *Urtica*	Label 3: *U. mem*	unknown	Label 1: *Parietaria*	Label 2: *Urtica*	Label 3: *U. mem*	unknown
VGG16	5	96	0	11	51	9	0	3
ResNet50	8	97	0	7	60	3	0	0

Supplementary Table S5 Average performance comparison of different models on 5-fold and 10-fold cross-validation subset selection, respectively. Standard deviation of five/ten subsets, is given in brackets.

	VGG16	VGG19	ResNet50	Flat model	Hierarchical model
Average performance of 5-fold cross-validation selection with Standard Deviation	0.958 (±0.007)	0.958 (±0.007)	0.975 (±0.002)	0.867 (±0.012)	0.891 (±0.023)
Average performance of 10-fold cross-validation selection with Standard Deviation	0.940 (±0.008)	0.938 (±0.008)	0.955 (±0.010)	0.840 (±0.019)	0.873 (±0.030)

Supplementary Table S6 Ablation study with ResNet50. The average performance of ResNet50 based on ten (about) 500-images subsets via 10-fold cross-validation selection method is given. Standard deviation, of ten subsets, is given in brackets. Numbers in italics refer to training without transfer learning and data augmentation, respectively.

	Training from scratch	With/*without* transfer learning	With/*without* data augmentation	With hard voting
Accuracy	0.793(±0.034)	0.919(±0.019)	0.933(±0.011)	0.955(±0.010)
	0.793(±0.034)	0.919(±0.019)	*0.919(±0.019)*	0.939(±0.024)
	0.793(±0.034)	*0.793(±0.034)*	0.804(±0.032)	0.841(±0.033)