

Aspects of the analysis of cell imagery: from shape to understanding Li, C.

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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	Montejaque (SP)	17/10/2011	54	WAG.1186948
judaica L.	Leiden, Stationsweg (NL)	19/11/2019	168	L.3993376*
(n = 1670)	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	Middelburg (NL)	26/06/2014	234	L.3974371
officinalis L.	Haarlem (NL)	13/07/2013	191	L.2073373
(n = 1359)	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.	Leiden, Hogeschool 1 (NL)	06/11/2019	316	L.3993380*
(n = 1055)	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	Amsterdam (NL)	11/2018	521	L.3993384*
Poir. ex Savigny	Overloon (NL)	17/06/2014	135	L.3959964
(n = 1118)	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.	Leiden (NL)	01/11/2019	128	L.3993386*
(n = 1270)	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* 

Image No.	Probability <i>Parietaria</i>	Probability <i>Urtica</i>	Probability <i>U.mem</i>	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

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

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 <i>Parietaria</i>	Probability <i>Urtica</i>	Probability <i>U.mem</i>	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 <i>Urtica</i>	Probability <i>U.mem</i>	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



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
Daviotaria	Parietaria Judaica	1670	8
Farlelaria	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

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

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	Label 1:	Label 2:	Label 3:	unknown	Label 1:	Label 2:	Label 3:	unknown
labels	Parietaria	Urtica	U. mem	unknown 1	Parietaria	Urtica	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.

	VCC16	VCC10	ResNet50	Flat	Hierarchical
	10010	10019		model	model
Average performance of 5-fold cross-	0.958	0.958	0.975	0.867	0.891
validation selection with Standard Deviation	(±0.007)	(±0.007)	(±0.002)	(±0.012)	(±0.023)
Average performance of 10-fold cross-	0.940	0.938	0.955	0.840	0.873
validation selection with Standard Deviation	$(\pm 0.008)$	(±0.008)	(±0.010)	(±0.019)	(±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	With/without	With/without data	With hard voting	
	scratch	transfer learning	augmentation		
	0.793(±0.034)	0.919(±0.019)	0.933(±0.011)	0.955(±0.010)	
Accuracy	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)	