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The wing interference patterns (WIPs) of *Parapanteles* (Braconidae, Microgastrinae): demonstrating a powerful and accessible tool for species-level identification of small and clear winged insects

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Abstract

Wing interference patterns (WIPs) are color patterns of insect wings caused by thin film interference. Thin film interference is the same phenomenon responsible for the refracted spectral colors sometimes visible on soap bubbles. Insect WIPs are static patterns due to the variable thickness of wing membranes and the colors produced depend on the thicknesses of wing membranes. While WIPs have been studied in several taxa of small insects, they have not been broadly adopted by insect taxonomists. We surveyed WIPs in one moderate-sized genus of parasitoid wasps, *Parapanteles* (Braconidae: Microgastrinae). Using an inexpensive microscope camera set-up and free imaging and analysis software, we detected consistent WIP differences between *Parapanteles* species. In some cases, WIPs can be used to diagnose sibling species that would otherwise require SEM images to differentiate or DNA barcodes. Wing interference patters are an underemployed character that may be similarly useful in many other taxa of small clear-winged insects.

Keywords

Braconidae, color patterns, Microgastrinae, *Parapanteles*, WIP, Wing interference patterns

Introduction

Wing interference patterns (WIPs), the rainbow colors that can appear on clear insect wings against dark background, have not been broadly adopted by insect taxonomists as morphological characters. Shevtsova et al. (2011) comprehensively investigated and called attention to these patterns, discovering that they are stable non-iridescent color patterns produced by thin film interference, where light that is reflected off of the upper or lower surface of a clear membrane constructively or destructively interferes with light approaching the membrane. The perceived color pattern is primarily caused by the varying thickness of the wing itself, and are, unlike iridescent colors from butterfly wing scales, static at a range of viewing angles (Shevtsova et al. 2011).

Wing interference patterns are under-used in species descriptions and as a tool for species-level identification of small clear-winged insects. Since their discovery as stable color patterns, they have rarely been reported in taxonomic works and even less frequently been used in species diagnoses or identification keys. In addition to discovering them, Shevtsova et al. (2011) comprehensively described the physical phenomenon that causes them and documented examples of WIPs in several Diptera and Hymenoptera taxa. Since then, WIPs have been documented in just 20 taxonomic or descriptive works (Hansson 2011; Shevtsova and Hansson 2011; Buffington and Sandler 2012; Hansson 2012; Hansson and Shevtsova 2012; Hernández-López et al. 2012; Simon 2012; Stigenberg 2012; Buffington and Condon 2013; Mitroiu 2013; Buffington and Forshage 2014; Drohojowska and Szwedo 2015; Zhang et al. 2014a, 2014b, 2016; Hosseini et al. 2019, 2020, 2021 2021; Pielowska-Ceranowska and Szwedo 2020; Butterworth et al. 2021; Conrow and Gelhaus 2022) and five experimental studies (Katayama et al. 2014; Brydegaard et al. 2018; Hawkes et al. 2019; Dong et al. 2020; White et al. 2021). Most of these studies focus on Hymenoptera (161 species), followed by Diptera (58 species), Hemiptera (8 species), and Odonata (1 species) (Suppl. material 1).

Because WIPs are a function of the varying thickness of wings, some authors have speculated that color may vary intraspecifically because overall wing thickness may be correlated to individual size (Shevtsova and Hansson 2011; Hernández-López et al. 2012). Therefore, they concluded that the colors of WIPs are less important than the patterns they form. Despite this, the majority of taxonomic works that document WIPs describe them in terms of qualitative colors and the relative portion of the wing those colors occupy (e.g., distal 1/3 magenta). Wing interference patterns have been used as characters in species diagnoses in only three publications to date (Hansson 2011; Shevtsova and Hansson 2011; Hansson and Shevtsova 2012), and have been used in a taxonomic key only three times (Mitroiu 2013; Zhang et al. 2014b; Hosseini et al. 2021). Efforts to quantify and compare WIPs have generally found them to be species-specific but rarely sexually dimorphic (Hawkes et al. 2019; Hosseini et al. 2019; Hosseini et al. 2020; Butterworth et al. 2021; White et al. 2021). To-date, WIPs have not been broadly adopted by taxonomists of small insects.

Microgastrinae (Hymenoptera: Braconidae) is a hyper-diverse subfamily of small parasitoid wasps that attack Lepidoptera (Mardulyn and Whitfield 1999). Microgastrinae currently has 2,999 described species (Fernández-Triana et al. 2020), representing roughly 5–10% of the estimated worldwide diversity of this group (Rodriguez et al. 2012; Fernández-Triana and Ward 2015). Their diminutive adult size and small number of morphological characters have made the generic-level taxonomy of this group difficult, and species-level diagnoses, absent DNA barcoding, often rely on subtly variable or minute characters that often require SEM imaging to observe (e.g., Valerio et al. 2009). Wing interference patterns have never been reported for microgastrines but are readily visible in living wasps in a container (DHJ, WH, personal communication).

Parapanteles Ashmead is a small genus of Microgastrinae with several species that are morphologically very similar to other genera (*Dolichogenidea* and *Glyptapanteles*) and frequently misdiagnosed (Valerio et al. 2009; Parks et al. 2020). Here, we document the WIPs of 7 described and 12 putative undescribed *Parapanteles* species from Costa Rica and Ecuador and present a simple and inexpensive method for quantifying and comparing WIPs that can contribute to identification keys and rapid species diagnosis.

Here we present the first study of WIPs in Microgastrinae (Hymenoptera: Braconidae), and an attempt to quantitatively compare the WIPs of closely related species using materials and methods already common in or freely available to most taxonomic laboratories that focus on small clear-winged insects.

Methods

Adult wasps used in this study were collected by two long-term Lepidoptera/parasitoid rearing projects: Área de Conservación Guanacaste (ACG) in Costa Rica (Janzen and Hallwachs 2009, 2016) and Yanayacu Biological Station in Ecuador (Dyer et al. 2017). A list of specimens used in this study is available in Table 1.

One set of fore and hind wings were removed from each adult wasp from samples stored in ethanol. Where available, wings from one male and one female per brood were removed and slide mounted on temporary slides. All species sampled are gregarious (i.e. the female lays multiple eggs in a single host) except *Parapanteles* sp. J and *Parapanteles* sp. K, which are solitary (i.e. females lay a single egg per host). We assume that all wasps eclosing from the cocoons from one caterpillar are siblings. Wings were sandwiched between two microscope slides which were taped together at the ends. This flattens wings more reliably than using a standard slide cover. As in Shevtsova and Hansson 2011, a drop of black India ink was spread on one slide to create a uniform black background behind the wings.

Wings were photographed at 50× magnification using a Cannon Rebel Xsi camera and an Amscope LED-144A-YK 144 LED ring light at maximum brightness. Wing images were not visually adjusted. Materials examined and qualitative descriptions of WIPs are available in Suppl. material 2. Images used in our analyses are available in Suppl. material 3.

Table 1. Materials examined for *Parapanteles* species included in this study from Área de Conservación Guanacaste (ACG), Costa Rica and Yanayacu Biological Station (YBS), Ecuador. Identification numbers for ACG specimens reflect voucher codes for COI DNA barcoding sequences on the Barcode of Life Database (BOLD).

Species	Source	ID #
Parapanteles continua	ACG, Costa Rica	DHJPAR0013724, DHJPAR0013810, DHJPAR0013718, DHJPAR0013733, DHJPAR0020230, DHJPAR0013716, DHJPAR0013725, DHJPAR0020228, DHJPAR0013723, DHJPAR0013717, DHJPAR0020236, DHJPAR0004196, DHJPAR0004192, DHJPAR0004189, DHJPAR0004190, DHJPAR0002808, DHJPAR0004798, DHJPAR0005102, DHJPAR0020859, DHJPAR0020911, DHJPAR0030974, DHJPAR0020231
Parapanteles em	ACG, Costa Rica	DHJPAR0004212, DHJPAR0004543, DHJPAR0004535, DHJPAR0004539, DHJPAR0002757, DHJPAR0020573, DHJPAR0020466, DHJPAR0020785, DHJPAR0020788, DHJPAR0020261, DHJPAR0002802
Parapanteles paradoxus	ACG, Costa Rica	DHJPAR0000248, DHJPAR0012335, DHJPAR0030924, DHJPAR0004544, DHJPAR0004209, DHJPAR0004534, DHJPAR0000246, DHJPAR0004194, DHJPAR0004541, DHJPAR0005103, DHJPAR0004796, DHJPAR0004800
Parapanteles sicpolus	ACG, Costa Rica	DHJPAR0004542, DHJPAR0000204, DHJPAR0000199, DHJPAR0004201, DHJPAR0004200, DHJPAR0004537, DHJPAR0004198, DHJPAR0004187
Parapanteles tessares	ACG, Costa Rica	DHJPAR0030744, DHJPAR0020916, DHJPAR0030733, DHJPAR0030762, DHJPAR0020905, DHJPAR0020850, DHJPAR0020849, DHJPAR0030752, DHJPAR0020904, DHJPAR0020852, DHJPAR0020857, DHJPAR0030773, DHJPAR0030975
Parapanteles tlinea	ACG, Costa Rica	DHJPAR0004188
Parapanteles sp. "valerio05"	ACG, Costa Rica	DHJPAR0020792, DHJPAR0012000, DHJPAR0020574, DHJPAR0020570, DHJPAR0020568, DHJPAR0020569, DHJPAR0031011
Parapanteles sp. "B"	YBS, Ecuador	45714, 26049, 37474, 20919, 24670
Parapanteles sp. "C"	YBS, Ecuador	12105, 45981, 48054
Parapanteles sp. "D"	YBS, Ecuador	8275, 35934, 37263, 37275, 37791, 44117
Parapanteles sp. "E"	YBS, Ecuador	36197, 36198, 36520
Parapanteles sp. "H"	YBS, Ecuador	2365, 2366, 2466, 4503
Parapanteles sp. "I"	YBS, Ecuador	42069, 43211, 46466, 66971
Parapanteles sp. "J"	YBS, Ecuador	27850, 27851, 34403, 34413, 36533
Parapanteles sp. "K"	YBS, Ecuador	28620, 32234, 36406, 36534, 38844

The average RGB (red, green, and blue) values of pixels in each fore wing image were measured using the "RGB Measure" feature in ImageJ v1.49 (Schneider et al. 2012). The value for each color component was divided by the average of all three average color values to calculate the relative "redness," "greenness," and "blueness" of each fore wing image (e.g., redness=R/((R+G+B)/3)). This averages out the contribution of black (R/G/B=0/0/0), white (R/G/B=255/255/255), and grey (R/G/B are all equal) pixels.

Arrays of relative redness, greenness, and blueness for each species were tested for normality in R v4.2.2 (R Core Team 2017) using the 'agricolae' and 'nortest' packages

(Gross and Ligges 2015; de Mendiburu and Yaseen 2020) via the Shapiro-Wilk test and for skewness, and then compared across species via ANOVA and Tukey's HSD test and visualized with ggplot2 (Beck 2017). Species with sample size lower than 3 were excluded from our statistical analysis. Data files and R code are available in Suppl. material 4.

Several metrics of fore wing size were measured to test whether they correlated with WIP patterns, because if they do then species-level differences in WIPs may simply be caused by some species being larger than others. Fore wing length (measured from the junction of C+Sc+R and M+Cu to the distal end of 3/M) and area were compared to each color array. In addition, overall fore wing shape was measured by dividing length by width (measured from the junction of r-rs and the stigma to the distal end of the anal lobe) to test if wing narrowness has any effect on wing thickness. Measurements were done in ImageJ v1.49 (Schneider et al. 2012) and tested for correlation via the Pearson Correlation test in R v4.2.2 (R Core Team 2017) using the 'hmisc' package (Harrell and Dupont 2019).

Linear discriminate function analyses were used to test how useful our quantification of microgastrine WIPs were by themselves for identifying species. Linear discrimination analyses were done in R v4.2.2 (R Core Team 2017). Several subsets of models were tested, and variables included the relative redness/greenness/blueness values for both fore wing and hind wing for all species, fore wing only for all species, hind wing only for all species, fore wing and hind wing data for each subclade containing two or more taxa, fore wing and hind wing data for species collected in the same country (Costa Rica or Ecuador), and fore wing and hind wing data for species that attack the same host family (Erebidae, Geometridae, Notodontidae, or Saturniidae). In each case 50% of the dataset was used to train the model and 50% of the dataset was used for validation. R code and data files are available in Suppl. material 4.

Results

Inter- and intraspecific variation in WIPs

The wing interference patterns of the species surveyed are generally consistent within species, although intraspecific consistency does vary. Both qualitatively (Suppl. material 3) and in terms of relative redness, greenness, and blueness (R.RGBs) (Table 2, Fig. 1), the species with purplish WIPs (*Parapanteles tessares*, *P. continua*, *P. sicpolus*, and *P.* sp. H) have the most consistent WIPs, while species with reddish or yellowish WIPs are more variable, especially *Parapanteles* sp. J and *Parapanteles* sp. K.

All R.RGB arrays were normally distributed except two *P. continua* arrays, one *Parapanteles* sp. E, one *P. paradoxus*, one *P. sicpolus*, and four *P. tessares* arrays (Table 2). The distributions of fore wing and hind wing R.RGBs among closely related species are often similar with one or two parameters significantly different (Fig. 1). For example, the R.RGBs of the sister species *P. tessares* and *P. continua* are not significantly different except for fore wing relative redness (higher in *P. continua*) and relative blueness

Table 2. Average relative redness (RR), greenness (RG), and blueness (RB) of the fore and hind wings of fifteen *Parapanteles* species plus or minus one standard deviation, with results of Tukey's HSD test, skewness, and Shapiro-Wilks' test for normality.

Wing	Species	n	Ave. RR	HSD	Skew	*P*-value	Ave. RG	HSD	Skew	P-value	Ave. RB	HSD	Skew	*P*-value
Fore	*Parapanteles continua*	41	1.05 ± 0.021	e	0.22	0.44	0.864 ± 0.018	bc	-1.19	**0.01**	1.086 ± 0.025	b	0.22	0.75
	Parapanteles em	16	1.149 ± 0.025	c	-0.52	0.60	0.836 ± 0.038	cd	1.21	0.09	1.016 ± 0.031	c	-0.22	0.96
	Parapanteles paradoxus	16	1.199 ± 0.023	ab	-0.29	0.91	0.912 ± 0.022	ab	-0.38	0.25	0.89 ± 0.033	f	0.43	**0.05**
	Parapanteles sicpulus	14	1.042 ± 0.013	ef	-0.78	0.25	0.884 ± 0.009	ab	0.59	0.55	1.074 ± 0.017	b	0.90	0.13
	Parapanteles sp. B	8	1.216 ± 0.029	a	-0.55	0.45	0.897 ± 0.051	ab	-0.75	0.10	0.887 ± 0.063	f	1.13	0.52
	Parapanteles sp. C	3	1.119 ± 0.018	cd	0.21	0.92	0.832 ± 0.018	cd	-0.72	0.73	1.049 ± 0.03	bc	-1.64	0.21
	Parapanteles sp. D	10	1.169 ± 0.029	bc	-0.15	0.86	0.834 ± 0.044	cd	0.57	0.54	0.997 ± 0.051	cd	0.59	0.50
	Parapanteles sp. E	3	1.183 ± 0.017	abc	0.51	0.81	0.915 ± 0.028	ab	-1.71	0.11	0.902 ± 0.013	ef	0.54	0.80
	Parapanteles sp. H	9	1.088 ± 0.022	d	1.13	0.19	0.813 ± 0.026	d	0.09	0.73	1.099 ± 0.039	b	-1.04	0.35
	Parapanteles sp. I	4	1.214 ± 0.05	ab	-0.71	0.87	0.929 ± 0.026	a	0.47	0.28	0.857 ± 0.037	f	0.01	0.06
	Parapanteles sp. J	5	1.212 ± 0.015	ab	1.07	0.50	0.887 ± 0.029	ab	0.03	0.25	0.9 ± 0.04	ef	-0.70	0.25
	Parapanteles sp. K	5	1.142 ± 0.078	c	-0.58	0.90	0.889 ± 0.026	ab	-0.17	0.42	0.968 ± 0.069	cde	1.52	0.25
	Parapanteles sp. valerio05	7	1.178 ± 0.032	abc	-0.98	0.44	0.868 ± 0.041	bc	0.33	0.41	0.954 ± 0.06	de	0.82	0.58
	Parapanteles tessares	25	1.012 ± 0.025	f	0.43	0.38	0.827 ± 0.025	cd	1.17	**0.01**	1.161 ± 0.036	a	-2.11	**0.00**
	Parapanteles tlinea	3	1.163 ± 0.026	bc	1.19	0.52	0.915 ± 0.026	ab	-1.57	0.28	0.922 ± 0.043	def	-1.05	0.58
Hind	*Parapanteles continua*	41	1.049 ± 0.026	f	0.03	0.84	0.89 ± 0.012	a	-0.63	0.31	1.062 ± 0.025	ab	1.02	**0.02**
	Parapanteles em	16	1.129 ± 0.023	cd	-0.60	0.29	0.84 ± 0.034	b	1.09	0.10	1.031 ± 0.046	bc	-0.54	0.39
	Parapanteles paradoxus	16	1.168 ± 0.026	bcd	1.06	0.26	0.863 ± 0.023	ab	0.53	0.71	0.969 ± 0.034	def	-0.14	0.70
	Parapanteles sicpulus	14	1.045 ± 0.012	f	2.16	**0.00**	0.894 ± 0.009	a	-0.64	0.47	1.062 ± 0.016	ab	-0.73	0.68
	Parapanteles sp. B	8	1.169 ± 0.032	abcd	0.04	1.00	0.872 ± 0.031	ab	0.24	0.23	0.959 ± 0.047	def	-0.82	0.11
	Parapanteles sp. C	3	1.106 ± 0.018	de	-0.69	0.74	0.839 ± 0.005	b	1.62	0.23	1.055 ± 0.016	abc	1.60	0.25
	Parapanteles sp. D	10	1.151 ± 0.042	bcd	0.14	0.87	0.835 ± 0.029	b	-0.09	0.35	1.014 ± 0.046	bcd	0.61	0.74
	Parapanteles sp. E	3	1.172 ± 0.052	abcd	1.73	**0.03**	0.895 ± 0.014	a	-1.48	0.35	0.933 ± 0.066	ef	-1.72	0.06
	Parapanteles sp. H	9	1.07 ± 0.014	ef	0.11	0.18	0.839 ± 0.018	b	0.09	1.00	1.091 ± 0.015	a	0.89	0.22
	Parapanteles sp. I	4	1.222 ± 0.043	a	1.23	0.55	0.875 ± 0.029	ab	-1.14	0.29	0.904 ± 0.065	f	-0.34	0.30
	Parapanteles sp. J	5	1.191 ± 0.039	ab	0.54	0.93	0.854 ± 0.043	ab	0.20	0.71	0.955 ± 0.078	def	-1.63	0.18
	Parapanteles sp. K	5	1.142 ± 0.041	bcd	0.72	0.81	0.867 ± 0.038	ab	0.40	0.41	0.99 ± 0.03	cde	0.19	0.27
	Parapanteles sp. valerio05	7	1.17 ± 0.033	abcd	0.22	0.75	0.891 ± 0.034	a	0.20	0.46	0.939 ± 0.06	ef	-0.34	0.73
	Parapanteles tessares	25	1.067 ± 0.019	ef	-0.58	**0.02**	0.853 ± 0.022	ab	1.46	**0.00**	1.081 ± 0.026	a	0.94	0.06
	Parapanteles tlinea	3	1.186 ± 0.039	abc	0.45	0.83	0.874 ± 0.033	ab	-1.12	0.55	0.94 ± 0.072	def	0.20	0.93

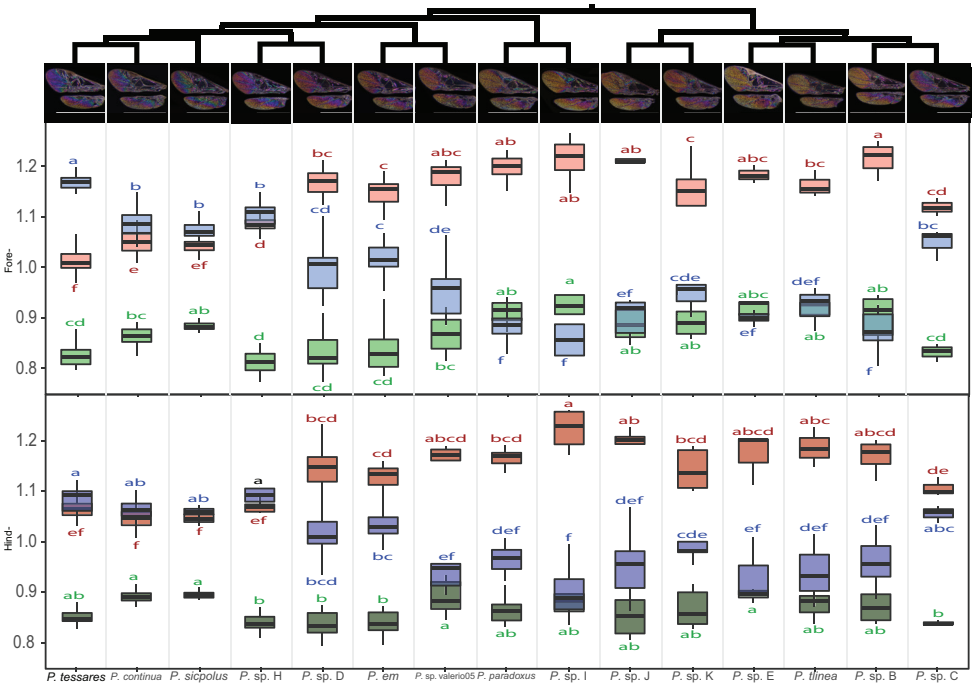

Figure 1. Box-and-whiskers plots of forewing and hind wing wing interference pattern relative rednesses (RR), greennesses (RG), and bluenesses (RB) shown in phylogenetic order. The cladogram above the figure is based on results from Parks et al. 2020. RR box-and-whiskers are shown in red, RB in blue, and RG in green (for colorblind: all RR values are greater than their corresponding RG values, so all red box-and-whisker plots are above green box-and-whisker plots in the figure). Results of Tukey's HSD test are displayed above or below each box-and-whisker. The white horizontal bar below each wing image represents 2 mm.

(higher in *P. tessares*), which corroborates the more uniformly purple appearance of *P. tessares*'s WIP.

We did not find evidence of sexual dimorphism in *Parapanteles* WIPs. Males and females of most species have similar WIPs, although in *Parapanteles* sp. D and *P. em* male WIPs are slightly more yellowish (Suppl. material 3: f and g.). Sexual dimorphism could not be assessed for 6 species: only females were available for *Parapanteles* sp. C, sp. J, sp. K, and sp. Valerio05, and only males were available for *Parapanteles* sp. I and sp. E.

Relative redness, greenness, and blueness and wing size

The majority of R.RGB arrays were not significantly correlated with wing length, area, or shape. Eleven of the 33 R.RGB tested were significantly correlated with wing length and 8 of 33 were significantly correlated with wing area. In each case the slope of the line of regression was slight and no R.RGB arrays were correlated with wing shape (Table 3).

Table 3. Average length, area, and shape (length/height) of the fore wings of fifteen *Parapanteles* species plus or minus one standard deviation, with coefficient of determination and the *p*-value of Pearson correlation tests of each measurement for each fore wing color array (relative redness (RR), greenness (RG), and blueness (RB)).

Species	n	Fore wing measurement	Average	*/RR r^2	*p*	*/RG r^2	*p*	*/RB r^2	*p*
Parapanteles continua	41	Length* (mm)	2.5 ± 0.18	0.09	0.06	0.16	**0.01**	0.29	**0.00**
		Height (mm)	0.67 ± 0.05	–	–	–	–	–	–
		Area* (mm^2)	0.67 ± 0.05	0.06	0.13	0.15	**0.01**	0.24	**0.00**
		Shape* (L/H)	3.76 ± 0.13	0.01	0.56	0.01	0.51	0.03	0.33
Parapanteles em	16	Length* (mm)	2.36 ± 0.21	0.45	**0.00**	0.79	**0.00**	0.29	**0.03**
		Height (mm)	0.64 ± 0.06	–	–	–	–	–	–
		Area* (mm^2)	0.64 ± 0.06	0.42	**0.01**	0.85	**0.00**	0.35	**0.02**
		Shape* (L/H)	3.71 ± 0.14	0.00	0.84	0.01	0.71	0.03	0.55
Parapanteles paradoxus	16	Length* (mm)	2.36 ± 0.21	0.08	0.28	0.04	0.49	0.01	0.76
		Height (mm)	0.62 ± 0.05	–	–	–	–	–	–
		Area* (mm^2)	0.62 ± 0.05	0.08	0.28	0.12	0.19	0.00	0.92
		Shape* (L/H)	3.81 ± 0.23	0.00	0.88	0.01	0.73	0.01	0.73
Parapanteles sicpulus	14	Length* (mm)	2.74 ± 0.13	0.24	0.08	0.07	0.34	0.24	0.07
		Height (mm)	0.74 ± 0.04	–	–	–	–	–	–
		Area* (mm^2)	0.74 ± 0.04	0.25	0.07	0.04	0.47	0.23	0.08
		Shape* (L/H)	3.7 ± 0.18	0.03	0.53	0.03	0.53	0.04	0.47
Parapanteles sp. B	8	Length* (mm)	2.11 ± 0.1	0.00	0.87	0.86	**0.00**	0.49	**0.05**
		Height (mm)	0.51 ± 0.03	–	–	–	–	–	–
		Area* (mm^2)	0.51 ± 0.03	0.08	0.49	0.69	**0.01**	0.28	0.18
		Shape* (L/H)	4.16 ± 0.15	0.37	0.11	0.04	0.65	0.18	0.30
Parapanteles sp. D	10	Length* (mm)	3.59 ± 0.15	0.00	0.95	0.66	**0.00**	0.48	**0.03**
		Height (mm)	0.93 ± 0.06	–	–	–	–	–	–
		Area* (mm^2)	0.93 ± 0.06	0.01	0.79	0.76	**0.00**	0.49	**0.02**
		Shape* (L/H)	3.88 ± 0.14	0.00	0.98	0.13	0.30	0.10	0.36
Parapanteles sp. H	9	Length* (mm)	3.11 ± 0.47	0.06	0.53	0.00	0.88	0.01	0.82
		Height (mm)	0.82 ± 0.13	–	–	–	–	–	–
		Area* (mm^2)	0.82 ± 0.13	0.06	0.51	0.00	**0.00**	0.02	0.72
		Shape* (L/H)	3.81 ± 0.08	0.10	0.42	0.37	0.08	0.34	0.10
Parapanteles sp. J	5	Length* (mm)	2.96 ± 0.21	0.02	0.82	0.02	0.83	0.00	0.95
		Height (mm)	0.78 ± 0.07	–	–	–	–	–	–
		Area* (mm^2)	0.78 ± 0.07	0.03	0.77	0.03	0.77	0.00	0.93
		Shape* (L/H)	3.81 ± 0.12	0.62	0.11	0.01	0.85	0.16	0.50
Parapanteles sp. K	5	Length* (mm)	2.66 ± 0.47	0.62	0.11	0.02	0.83	0.74	0.06
		Height (mm)	0.7 ± 0.12	–	–	–	–	–	–
		Area* (mm^2)	0.7 ± 0.12	0.09	0.16	0.02	0.82	0.61	0.12
		Shape* (L/H)	3.81 ± 0.17	0.16	0.51	0.05	0.71	0.29	0.35
Parapanteles sp. valerio05	7	Length* (mm)	2.4 ± 0.13	0.07	0.58	0.48	0.09	0.10	0.48
		Height (mm)	0.62 ± 0.04	–	–	–	–	–	–
		Area* (mm^2)	0.62 ± 0.04	0.02	0.77	0.55	0.06	0.18	0.35
		Shape* (L/H)	3.87 ± 0.2	0.30	0.20	0.10	0.49	0.25	0.25
Parapanteles tessares	25	Length* (mm)	2.33 ± 0.09	0.04	0.37	0.18	0.03	0.18	0.03
		Height (mm)	0.61 ± 0.04	–	–	–	–	–	–
		Area* (mm^2)	0.61 ± 0.04	0.00	0.89	0.13	0.08	0.07	0.19
		Shape* (L/H)	3.83 ± 0.15	0.00	0.91	0.00	0.73	0.00	0.78

Linear discriminate function analysis

Results for linear discriminate function analyses varied widely and are available in Suppl. material 4. Linear discriminate function analysis using our complete dataset predicted species accurately only 34% of the time, but was more accurate with some subsets of species separated by subclade, geography, or host use (e.g., species prediction of species found in Costa Rica was 83% and species parasitizing saturniids was 75%).

Discussion

The wing interference patterns of *Parapanteles* are consistent within species and distinct between species, often enough to be diagnostic by themselves. Among the species surveyed, the WIPs of *Parapanteles tessares*, *P. continua*, *P. sicpolus*, *P.* sp. H, and *P.* sp. C were the most distinct. These species tended to have more green and purple in their WIPs, while the remaining species' WIPs were predominantly red and/or yellow.

Wing interference patterns are directly related to the thickness of wing membranes, and previous publications have speculated that WIP colors should change as individuals get larger because cuticle thickness may increase with body size (Shevtsova and Hansson 2011; Hernández-López et al. 2012). We are not aware of any studies investigating the allometry of body or wing cuticle thickness. Among the species we surveyed, some relative redness, greenness, and/or blueness arrays were significantly correlated with wing size and/or area in some species, but in each of these cases the slope of the corresponding linear regression was very slight (Table 3). Correlation with wing size (as a proxy for body size) alone does not account for the differences between the WIPs of closely related *Parapanteles* species. We were not able to use WIPs alone to reliably predict the identity of an unknown specimen from a large number of species, but were able to discriminate between species in some subclades or subsets of species defined by location or host use (Suppl. material 4). Wing interference patterns are not likely to be useful for automated species identification for many taxa, but are useful as an additional and generally overlooked morphological character to be used in conjunction with other characters for species diagnosis, as any morphological character traditionally would be. When viewed this way they are often one of the most conspicuous and accessible morphological characters of the physically small taxa on which they appear.

Wing interference patterns are directly related to the wavelength of the light passing through the wing membrane, which is a major weakness for using any measurement derived from RGB values for diagnostic purposes. The relative RGB values we measured in this study were not consistent if the wing was illuminated with a different light source. This limitation can be solved by using a consistent light source, and the light source which we used for all WIP photographs in this study, an Amscope LED-144A-YK 144 LED ring light, is widely available and relatively inexpensive. Using one or more lasers of specific wavelengths to illuminate WIPs could offer a more replicable

and standardizable method for documenting WIPs, although using one or a few wavelengths would result in less data than full spectrum white light. Wing interference patterns can be observed *in situ* on pinned specimens, but these are of little use compared to WIPs observed on slide-mounted wings. Including WIP slides (wing slides with India Ink painted on the back) of at least a few paratype individuals with the type series of small winged insects would ameliorate most of the problem posed by variations between light sources, and expand the usefulness of WIPs for future studies.

Experiments in *Drosophila* have repeatedly shown WIPs to be subject to sexual selection (Katayama et al. 2014; Hawkes et al. 2019). While this has not been experimentally tested in other taxa, this and other studies have found that WIPs are frequently species-specific (Shevtsova et al. 2011; Buffington and Sandler 2012; Zhang et al. 2014b, 2016; Hosseini et al. 2019; Butterworth et al. 2021; Hosseini et al. 2021). Similarly to the *Drosophila* species used in the sexual selection experiments, microgastrinae males also display their wings to females during courtship (Bredlau and Kester 2019). The colors of WIPs are visible *in situ* and in natural settings whenever insect wings are displayed in front of a dark background (e.g. green leaves), and the colors that compose them occur in spectra visible to most insects (Shevtsova et al. 2011; Brydegaard et al. 2018; Butterworth et al. 2021). Anecdotally, we found that closely related sympatric species tended to be more subjectively different (i.e. ((*Parapanteles tessares*, *P. continua*), *P. sicpolus*) and (*P. em*, *P.* valerio05) from Costa Rica and (*P.* sp. B, *P.* sp. C) from Ecuador), while closely related allopatric species tended to be less distinct (i.e. (*P. paradoxus*, *P.* sp. I) and (*P.* sp. E, *P. tlinea*) (Fig. 2). This suggests that WIPs may be used by microgastrines for conspecific recognition, but this is entirely speculative and would require a broader survey of microgastrine WIPs to test. We only included two solitary species (i.e. females oviposit a single egg into each host, *P.* sp. J and *P.* sp. K) in our study. These two species had the most variable WIPs and wing sizes. The relationship to host quality and adult wasp size may be more direct in solitary

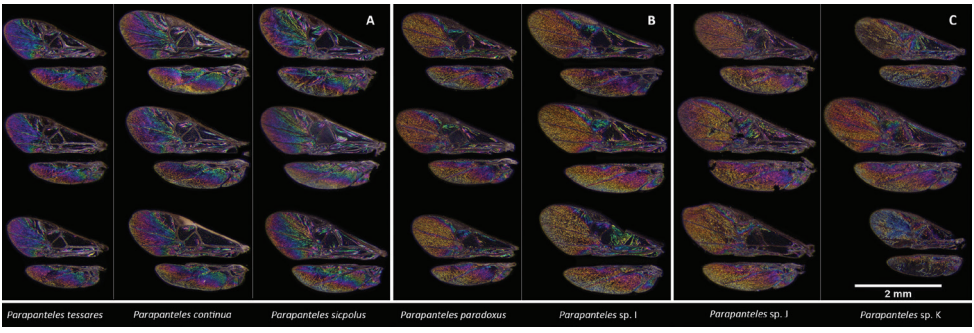

Figure 2. Right wings of three different individuals from seven *Parapanteles* species showing wing interference patterns. A shows three gregarious sympatric sister species ((*P. tessares*, *P. continua*), *P. sicpolus*) from Area de Conservación Guanacaste (ACG) in Costa Rica. B shows two gregarious allopatric sister species, one from AVG (*P. paradoxus*) and one from Yanayacu Biological Station in Ecuador (*P.* sp. I). C shows two solitary sister species from Yanayacu Biological Station.

species that use small host caterpillars than gregarious species attacking larger caterpillars. In such solitary species, poor quality hosts may have less resources available for parasitoids and result in smaller adults, while gregarious species can oviposit fewer eggs to account for poor quality hosts which may result in more consistent adult wasp sizes. Even so, *P.* sp. J fore wings are significantly redder than *P.* sp. K (Figs 1, 2).

Conclusions

In general, WIPs can be observed and documented with very little additional effort for most taxonomists who work on small winged insects. We predict that they can be a large source of new morphological characters for the taxonomy and systematics of these tiny animals. The only materials required are a dissecting microscope with a camera attachment, a ring light, glass slides, and India Ink. Wing interference patterns are often species-specific and useful for *Parapanteles* wasps, and will likely be for most other microgastrine wasps.

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Supplementary material 1

Taxonomic summary of published wing interference pattern images and/or descriptions

Authors: Shuyang Jin, Kyle S. Parks, Daniel H. Janzen, Winnie Hallwachs, Lee A. Dyer, James B. Whitfield

Data type: xlsx

Link: https://doi.org/10.3897/jhr.96.111382.suppl1

Supplementary material 2

Qualitative descriptions and materials examined for *Parapanteles* species included in this study

Authors: Shuyang Jin, Kyle S. Parks, Daniel H. Janzen, Winnie Hallwachs, Lee A. Dyer, James B. Whitfield

Data type: docx

Link: https://doi.org/10.3897/jhr.96.111382.suppl2

Supplementary material 3

Wing interference patterns

Authors: Shuyang Jin, Kyle S. Parks, Daniel H. Janzen, Winnie Hallwachs, Lee A. Dyer, James B. Whitfield

Data type: zip

Explanation note: Wing interference patterns of *Parapanteles tessares* (a), *P. continua* (b and c), *P. sicpolus* (d), *P.* sp. H (e), *P.* sp. D (f), *P. em* (g), *P.* sp. valerio05 (h), *P. paradoxus* (i), *P.* sp. I (j), *P.* sp. J (k), *P.* sp. K (l), *P.* sp. E (m), *P. tlinea* (n), *P.* sp. B (o), and *P.* sp. C (p). Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

Link: https://doi.org/10.3897/jhr.96.111382.suppl3

Supplementary material 4

Data files and R code

Authors: Shuyang Jin, Kyle S. Parks, Daniel H. Janzen, Winnie Hallwachs, Lee A. Dyer, James B. Whitfield

Data type: zip

Explanation note: Data files and R code used to calculate mean, standard deviation, ANOVA, Tukey's HSD, Skewness, Shapiro-Wilks normality test, and linear discriminate functions analysis of forewing and hindwing relative redness, greenness, and blueness, and Pearson's correlation of forewing length, forewing area, and forewing shape (H/L) to forewing relative redness, greenness, and blueness.

Link: https://doi.org/10.3897/jhr.96.111382.suppl4