

Wing Morphology and Echolocation of *Rhinopoma hardwickii* (Lesser Mouse-tailed Bat, Gray, 1831)

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Abstract: *Rhinopoma hardwickii* is currently classified as a member of the Yinpterochiroptera suborder, which includes frugivorous and some insectivorous bats. This species is the smallest in the Rhinopomatidae family and easily identified by its long tail. The wing morphology and echolocation calls of this species were studied to see if there were any changes in wing morphology between sexes, echolocation calls across different environments such as natural (roost and field) and controlled (captive), as well as different geographical areas. In this study, a total of 41 individuals (27 male and 14 female) of *R. hardwickii* were captured and their wing morphology was measured. The results show that there were no statistically significant variations in their morphometric characteristics or in wing morphology between the sexes. This species has with high wing loading and a high aspect ratio, as well as pointed wing tips. The echolocation calls consisted up to five harmonics of FM and CF-FM sweeps. Peak frequencies, start frequency, end frequency, and IPI of three separate environment parameters (roost, captive, and field recording) differed significantly ($p > 0.001$). Moreover, we compared the frequency at maximum energy with four different geographical regions such as Kerala, Gujarat, and Israel to current study, and found that the frequencies of bat calls do not vary with geographical region ($H=0.667$, $df=3$, $p=0.881$). Therefore, the current study provides accurate identification of *R. hardwickii* on the basis of echolocation call in a different environment. The echolocation call and wing morphology data clearly show that this species is a fast flyer with limited manoeuvrability that feeds on forest canopy or over water bodies.

Keywords: Echolocation, frequency, rhinopoma, morphology, multi-harmonic, wing.

1. Introduction

Members of Rhinopomatidae family are insectivorous bats native to Old-World tropics that live primarily in deserts and steppes. Rhinopomatidae is a monotypic family with a single known genus, *Rhinopoma* (Geoffroy, 1818), with five species: *Rhinopoma hardwickii* (Gray, 1831), *R. microphyllum* (Brünnich, 1782), *R. muscatellum* (Thomas, 1903), *R. cystops* (Thomas, 1903) and *R. macinnesi* (Hayman, 1937). *Rhinopoma hardwickii*, commonly known as the Lesser Mouse-Tailed Bat or Long-Tailed Bat, was named after Major General Thomas Hardwicke (1755–1835), an English soldier and naturalist who served many years in India. These bats are smallest in their family and are easily identified by their long tail. They primarily roost in natural caves, monuments, abandoned buildings, dry bushes, rocky places, and barren mountains (Prakash, 1961; Benda et al., 2004). Their colony consists of hundreds to thousands of individuals (Purohit and Senacha, 2004; Benda et al., 2004). They also share colonies with *Taphozous* spp. (Singh et al., 2021). They are distributed over a wide range of geographic areas from Morocco, Senegal, and Kenya, as well as Arabia and the Middle East, India, Thailand, and the Sudan Archipelago (Van Cakenberghe and De Vree, 1994; Hill,

1977). The Rhinopomatidae family has been classified into a new suborder, Yinpterochiroptera (Springer, 2001) along with Pteropodidae, Rhinolophidae, Hipposideridae, Megadermatidae and Craseonycteridae (Hulva and Horacek, 2002; Teeling et al., 2002; Van Den Bussche and Hofer, 2004). Simmons and Stein (1980) reported that *R. hardwickii* as a primitive echolocating bat, however, Habersetzer (1981) research revealed that *R. hardwickii* possesses a complex echolocating system that produces at least two distinct types of sounds: Frequency Modulated (FM)-sounds are produced when approaching an obstacle and during cluster outfly, while Constant Frequency (CF) sounds are generated in the open by both solitary and group flying bats.

Few studies have been conducted on the echolocation of *R. hardwickii* (Hackett et al., 2016; Srinivasullu and Srinivasullu, 2017; Shah and Srinivasullu, 2020), but none have taken into account changes in habitats such as the captive, field and roost recording. In addition to echolocation, wing morphology is a key element in determining the behavior of any bat species (Norberg and Rayner, 1987; Pennycuik 1989). Except for Norberg and Rayner (1987), no information on the wing morphology of *R. hardwickii* is currently available. There is a possibility of species misidentification due to lack of bat call data accessible for comparative purposes, particularly survey calls (Duffy et al., 2000; Gannon et al., 2004). Many factors contribute for the intraspecific call variability, including age (Jones and Kokurewicz, 1994),

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individual identity (Masters et al., 1995), foraging mode (Griffin et al., 1960), flight situation (Berger-Tal et al., 2008), acoustic clutter (Broders et al., 2004), ambient noise (Gillam and McCracken, 2007) and presence of conspecifics (Chiu et al., 2009). The problem became much more complicated when regional call comparisons were not performed (Thomas et al., 1987; Barclay et al., 1999; O'Farrell et al., 2000; Reinhold et al., 2001; Law et al., 2002).

Therefore, the following questions were arising: (1) are there any differences in wing morphology between sexes, (2) are there any difference in echolocation calls between natural (field and roost recording) and controlled (captive), and (3) are there any differences in echolocation calls between different geographical areas (frequency at maximum energy; FMaxE). FMaxE, which occurs in the outward pulse of a call, is regarded one of the most consistent and crucial echolocation call parameters (Fullard et al., 1991). The study was conducted to fill the current knowledge gap, considering the three questions listed above.

2. Materials and Methods

Field Survey and Identification of Bats

Field surveys were carried out from April 2019 to February 2020. Bats were captured for individual identification at their roosting sites using nylon mist nets (9.0 m X 2.0 m, with mesh size 38.0 mm, Avinet, Dryden, USA), and hoop net. Maps of roosting sites were prepared using Arc GIS. Morphological measurements of adult bats such as body length, forearm length, hind arm, metacarpals, tail, head length and wingspan etc. were taken using digital venire calipers (Mitutoyo, Japan), and body mass was measured with electronic balances (ACCULAB Sartorius group, EC-211). Bats were recognized using morphological criteria, as described by Bates and Harrison (1997). Bats were captured and handled in accordance with the guidelines of the American Society of Mammologists (Sikes et al., 2011) and relevant ethical permits were secured for data collection vide Letter No. 214/11/DAAS/BBAU/2011 of Babasaheb Bhimrao Ambedkar University and Archaeological Survey of India, Lucknow circle for bat survey (F. No. 10-16/23/2013-M 11535).

Wing Morphology and Call Analysis

The bat was positioned on its ventral side on a large graph paper, with their wings stretched to their leading-edge perpendicular to the body axis. The wing outlines were then traced onto the graph paper and the area of wings was assessed. Arm length, wing length (cm) and hand wing (cm), arm wing area (cm²) and hand wing area (cm²), wing span (cm) and wing area (cm²) were also measured, as reported (Norberg and Rayner 1987). The wing loading (WL), aspect ratio (AR), and tip shape index (TSI) were estimated as per the Norberg and Rayner (1987) and Pennycuik (1989) reports. Relative wing loadings (RWL) were estimated using Norberg et al. (2000).

The echolocation calls of *R. hardwickii* were recorded at four separate places (Fig. 1) in three environments: roost, field and captivity. The conditions for three separate ecosystems were as follows.

- a) Roost: bat detector was placed on unattended mode overnight to record calls under the roosts.
- b) In the field: manual recoding was performed at the moment of emergence near the roost in open ground.
- c) Captive: Individuals of this species were captured using mist and hoop nets, then released in a room (10X10 feet). Once relaxed, the recorder was set to unattended mode for at least 2 hours per recoding.

The calls were recorded using a heterodyne in an unattended mode with time expansion (10X). The fast fourier transform (FFT) size was 512 samples with Hanning window. Each 20 sec recording lasted 2 min and included a maximum of five strings at a five-minute interval. The bat calls were analyzed using Bat sound Pro analysis software (version 2.1), which yielded the following call parameters: peak frequency, start and end frequencies, call duration, inter-pulse interval, band width, maximum and minimum frequency. We also compare the echolocation call of *R. hardwickii* with previous studies (Hackett et al., 2016; Srinivasullu and Srinivasullu, 2017; Shah and Srinivasullu, 2020) and the current study in Uttar Pradesh, which are from different geographical areas (Fig. 2, Table 4).

Statistical Analysis

The data was analyzed using SPSS 21.0 (SPSS Inc, USA). The distribution of data sets was analysed using a descriptive statistic, with normality and homogeneity being considered. $P < 0.05$ was considered for normally distributed data sets. All morphological data were abnormally distributed ($p > 0.05$), so the Kruskal Wallis H-test was applied to see whether there were any statistical variations in wing morphology between sexes.

3. Results

A total of 41 *R. hardwickii* individuals (27 male and 14 female) of were captured from their day roost utilizing hoop and mist nest. Table 1 shows the morphological measurements of *R. hardwickii*. Males had little higher body mass (20.71 ± 2.95 g) then females (19.86 ± 3.38 g) but there was no significant differenc in weight ($H = 0.41$, $p=0.522$). Females had somewhat larger forearms (60.73 ± 1.62) than males (58.83 ± 2.26 mm), although the differences was not significant ($H=0.126$, $p=0.723$). There were no statistical significance differences in arm wing length, hand wing length, arm wing area, hand wing area, aspect ratio, tip length ratio, wing-loading and relative wing loading between the sexes ($p > 0.05$, Table 2). Therefore, we pooled the wing morphology data of

males and females. The average wing loading of *R. hardwickii* was 11.92 ± 1.87 (N/m²) with an aspect ratio was 7.04 ± 0.44 . The relative wing loading for *R. hardwickii* was 8.41 ± 5.57 . The tip length ratio and tip area ratios were 0.68 ± 0.04 and 0.44 ± 0.05 , respectively. The tip shape index was measured and found 1.57 ± 0.15 .

We recorded 5330 *R. hardwickii* calls throughout a 15-hour 50-minutes period at four sites, and 167 of them were selected for analysis. The calls consisted up to five harmonics of FM and CF-FM sweeps, with the fourth harmonic being the most common; the second harmonic was the most strong. The second harmonic's peak frequency is 28.8 – 38.7, the third harmonic is 37.9 – 50.6, and the fourth harmonic 42.7 – 62.7 kHz. The Frequency at maximum energy (FMaxE) ranged from 28.8 to 38.7 (33.23 ± 2.38) kHz. The start frequency ranged from 19.90 to 36.90 kHz (30.78 ± 3.04), and the end frequency ranged from 21.4 to 38.8 kHz (32.15 ± 2.84). The pulse interval was 39.92 ± 46.86 ms. When we analysed start frequency, end frequency and peak frequency along with Inter Pulse Interval (IPI) at three different sites (roost, captive and field recording), we found that there were significant differences between captive, field and roost sites ($p=0.001$, Table 3). When we compared the frequency at maximum energy of *R. hardwickii* to four different geographical regions, namely Kerala, Gujrat, and Israel, with the current study, we found that the frequency did not vary by geographical region ($H=0.667$, $df=3$, $p=0.881$) (Table 4).

4. Discussion

The current study reveals that *R. hardwickii* is a fast flyer with poor maneuverability that feeds on forest canopy or over water body, has a larger aspect ratio ($AR > 8.0$), a short wing span, high wing loading, average relative wing loading, and a small surface area. Bats with high AR and RWL are fast flyer but have poor flight maneuverability (Freeman, 1981), and they feed in open unobstructed environment such as over forest canopy or over water (Marinello and Bernard, 2014). Kingdon (1974), Smith and Starrett (1979) report rhinopomatids foraging in open ground, apart from impediments, at relatively high altitude, but Neuweiler (1984), Habersetzer (1986) describe their fly at intermediate heights in open spaces of forest around the canopy, but below the high and fast flyer Emballonuridae and Molossidae in the same habitat. *Rhinopoma hardwickii* have a series of alternating flutters and glides, with a rising and falling motion (Harrison, 1964).

Previously, only five studies on *R. hardwickii* echolocation calls were conducted, some of which were incomplete (Chaturvedi et al., 2018), and no one considered the wing morphology, despite the fact that wing morphology is directly related to echolocation and feeding behavior in all insectivorous bat species.

Rhinopoma hardwickii produces narrowband, multi harmonic signals with CF/FM, which are dominated by harmonic other than

fundamental harmonic, as supported by other authors (Habersetzer, 1981; Jones and Teeling, 2006). The narrow broadband call with low frequency is associated with high wing loading and high aspect ratio reported in fast flying, open area foraging species (Norberg and Rayner, 1987); consequently, the foraging result clearly show that *R. hardwickii* is fast flyer who forages in open space. Many authors also reported that this species generates multi harmonic calls with CF/FM (Srinivasullu and Srinivasullu, 2017; Shah and Srinivasullu, 2020), quasi constant frequency (QCF) calls (Hackett et al., 2016), second harmonics with maximal energy. The call frequency of *R. hardwickii* differs from previous findings, which ranges from 32–35 kHz (Hackett et al. (2016), 31.3 - 33.6 kHz (Srinivasulu and Srinivasulu 2017), 30.90 - 32.25 kHz (Shah and Srinivasulu 2020) while the current result shows the 28.8 – 38.7 kHz, that's is approx. 2 kHz less than minimum call frequency and 3 kHz higher call frequency in previous reports. Lower frequency calls with narrow band widths are required for long range detection of prey, which is characteristic feature of fast flyer species (Neuweiler, 1984), these assertions supports the current finding.

All of the author mentioned did not investigate the differences in *R. hardwickii* echolocation calls in different environments, such as natural (field and roost), controlled (captive), although the result of this study clearly indicated that the *R. hardwickii* has varied call frequencies at different habitats. Habersetzer (1981) also reported that *R. hardwickii* has complex echolocating system which alters the form of signals in different situation. The result of current study, which is also supported by many authors, found that individuals of bats species changes their calls in response to changing habitats as well as distance from obstacles (Kalko and Schnitzler, 1993; Bartonicka and Rehak, 2005) or proximity to other bats (Obriest, 1995; Ratcliffe et al., 2004; Ulanovsky et al., 2004; Gillam et al., 2007; Bates et al., 2008; Amichai et al., 2015).

This study found that the call frequency and structure of *R. hardwickii* did not vary by geographical regions, however, some authors reported that the populations of same species in different geographical region may have different call structures (Thomas et al., 1987 and Murray et al., 2001).

5. Conclusion

The result of current study found that there were no differences in wing morphology between the sexes. *Rhinopoma hardwickii*'s echolocation calls vary according to situation, such as natural (roost and field recording) and controlled (captive), but do not change with geographical regions. The wing morphology data clearly indicates that *R. hardwickii* is fast flyer with limited maneuverability, and this species feeds on forest canopy or over water bodies. The data provided in this study may provide to other researchers in accurately identifying of *R. hardwickii* based on echolocation calls in various habitats, whereas wing morphology indicates foraging nature.

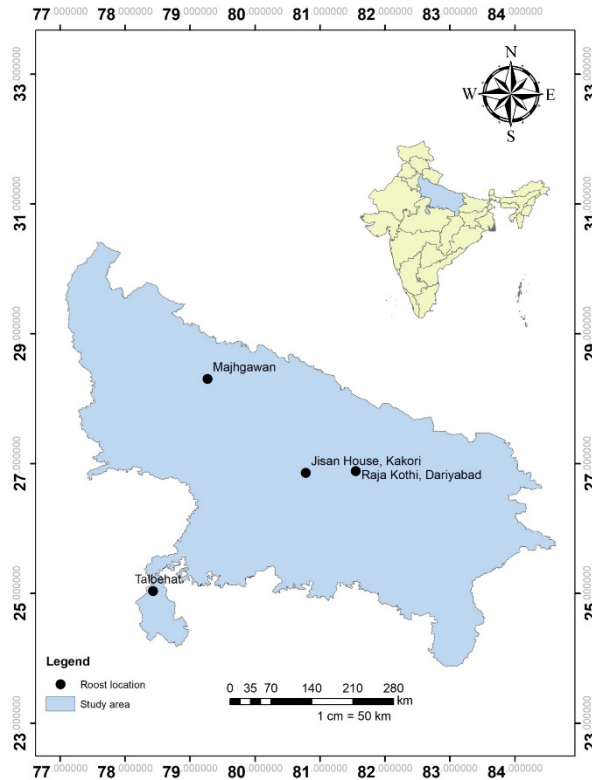


Figure 1. The roosts location of *R. hardwickii* in Uttar Pradesh, India.

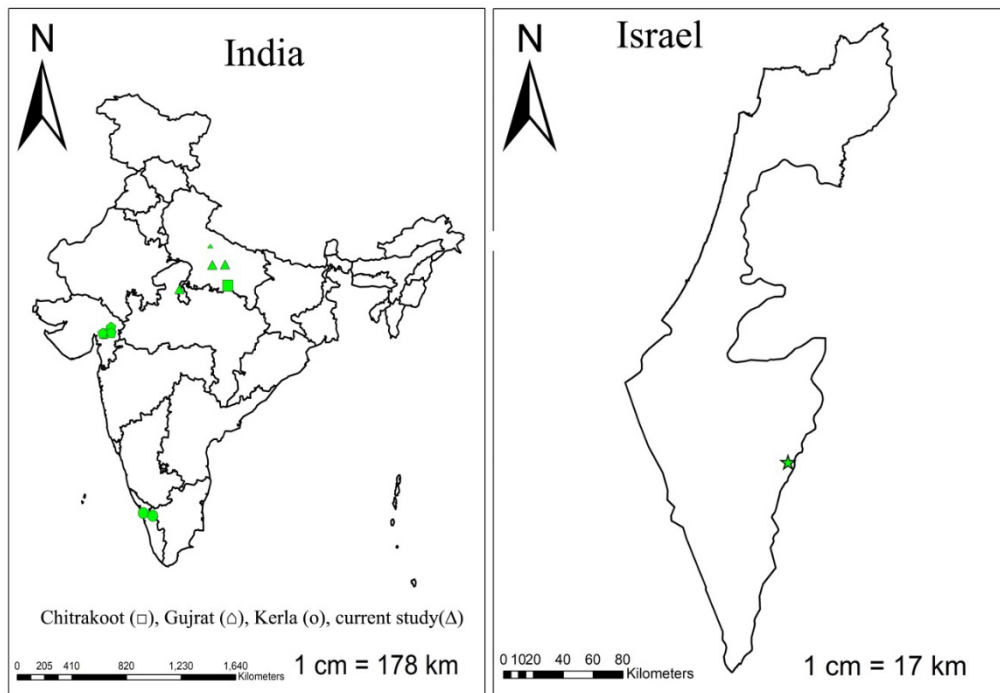


Figure 2. The echolocation calls of *R. hardwickii* recorded from various geographical regions. Right panel map shows location within India (Chitrakoot, Gujrat, Kerala) whereas, left panel indicates location in Israel.

Table 1. Morphological measurements of males and females *R. hardwickii* in millimetre (mm), unless otherwise mentioned. Values are given Mean ± SD.

Parameter	Male (n=27)	Female (n=14)	H value	P value
Body mass (g)	20.71 ± 2.95	19.86 ± 3.38	0.41	0.522
BH length	71.46 ± 3.69	71.12 ± 3.39	0.261	0.61
Head length	19.71 ± 4.33	23.05 ± 1.25	2.847	0.092
Tail length	58.37 ± 6.49	60.73 ± 6.34	1.557	0.212
Ear length	14.23 ± 1.44	13.96 ± 1.13	0.001	0.974
Ear width	8.96 ± 1.09	8.09 ± 1.11	3.031	0.082
Targus	5.90 ± 0.81	5.82 ± 0.39	0.39	0.532
Forearm length	58.83 ± 2.26	60.01 ± 1.62	0.126	0.723
5th Metacarpal	43.54 ± 1.53	42.60 ± 1.43	2.185	0.139
First phalanx	10.80 ± 0.59	10.85 ± 0.69	0.002	0.964
Second phalanx	9.52 ± 0.86	9.28 ± 0.42	1.155	0.282
4th Metacarpal	38.26 ± 1.67	36.66 ± 1.70	5.013	0.025
First phalanx	12.87 ± 0.67	13.32 ± 0.40	2.187	0.139
Second phalanx	10.55 ± 1.63	10.98 ± 0.13	0.032	0.858
3rd Metacarpal	44.05 ± 2.26	40.82 ± 1.65	5.43	0.2
First phalanx	8.76 ± 0.75	9.03 ± 1.32	0.98	0.754
Second phalanx	17.72 ± 1.06	18.10 ± 0.41	0.393	0.531
2nd Metacarpal	43.47 ± 2.26	42.08 ± 1.10	2.896	0.89
Thumb length	7.00 ± 0.49	6.46 ± 0.59	7.967	0.005
Hind arm	30.19 ± 1.75	29.76 ± 1.11	1.287	0.257
Foot	7.47 ± 3.10	8.30 ± 3.74	0.586	0.444
Body width	28.85 ± 7.93	25.83 ± 10.17	1.001	0.317
Wing span (cm)	20.51 ± 6.18	29.88 ± 0.87	3.509	0.061

Table 2. Wing morphology of male and female *Rhinopoma hardwickii*. Values are given Mean ± SD.

Wing characteristics	Male (n = 27)	Female (n = 14)	H	P
Arm Wing Length	10.23 ± 0.34	10.20 ± 0.42	0.077	0.782
Arm Wing Area	58.35 ± 6.63	58.96 ± 3.59	0.049	0.826
Hand Wing Length	6.90 ± 0.33	6.99 ± 0.34	0.292	0.589
Hand Wing Area	25.22 ± 1.84	25.80 ± 2.29	0.291	0.590
Wing Area	1.67 ± 0.14	1.70 ± 0.11	0.206	0.650
Tip Length Ratio	0.68 ± 0.04	0.69 ± 0.04	0.219	0.640
Tip Aspect Ratio	0.44 ± 0.06	0.44 ± 0.03	0.027	0.869
Tip shape index	1.56 ± 0.17	1.57 ± 0.09	0.170	0.680
Wing Loading	12.27 ± 1.83	11.23 ± 1.82	2.676	0.102
Relative Wing loading	8.83 ± 2.54	7.60 ± 2.51	2.287	0.130
Aspect Ratio	7.06 ± 0.47	6.99 ± 0.39	0.076	0.783

H= Kruskal – Wallis H test

Table 3. Echolocation calls of *R. hardwickii* in three different habitats. Values were presented in Mean ± SD.

Echolocation	Captive (n =94)	Field (n =35)	Roost (n = 38)	H*	p – value
Start Freq.	31.04 ± 2.19	26.81 ± 1.53	29.39 ± 0.97	80.165	0.001
End Freq.	34.77 ± 1.71	30.83 ± 1.21	36.12 ± 1.34	85.403	0.001
Peak Freq.	32.83 ± 2.13	30.04 ± 0.81	32.76 ± 0.87	101.791	0.001
I.P.I. (ms.)	38.75 ± 7.59	112.09 ± 49.76	24.00 ± 16.56	64.401	0.001
Bandwidth	3.73 ± 1.71	4.02 ± 1.08	6.72 ± 1.18	81.245	0.001

Freq. = Frequency, all frequency in KHz, H = Value of Kruskal Wallis H – test

Table 4. Comparison of wing morphology and Echolocation of *Rhinopoma hardwickii* bats with other studies, frequencies were given in kHz, pulse duration and pulse interval were given in millisecond (ms.). Values are given in Mean ± SD

Parameters/ Studies	Habersetzer, 1981 (n =)	Hackett et al., 2016 (*n =257/3)	Srinivasulu & Srinivasulu, 2017 (*n=1/12)	Chaturvedi et al. 2018 (*NM)	Shah and Srinivasulu, 2020 (*n=58)	Present study (*n = /167)	
Echolocation calls	Peak Frequency	30	33.99 ± 1.13	32.48 ± 0.75	32	31.32 ± 0.93	33.23 ± 2.38
	Start frequency	32.50	35.85 ± 1.81	37.05 ± 0.46	-	34.52 ± 2.40	32.15 ± 2.84
	End frequency	35.0	32.72 ± 1.39	26.66 ± 0.94	-	28.32 ± 3.58	30.78 ± 3.04
	Pulse duration		8.57 ± 1.28	2.21 ± 0.44	-	3.86 ± 1.48	
	Pulse interval		-	-	-		39.92 ± 46.86
	Band width						4.47 ± 1.78
Wing morphology (n= 41)	Wing span (cm)	-	-	-	-	-	34.31 ± 0.96
	Wing area (×10 ⁻³ m ²)	-	-	-	-	-	1.68 ± 0.13
	Aspect ratio	-	-	-	-	-	7.04 ± 0.44
	Wing loading (N/m ²)	-	-	-	-	-	11.92 ± 1.87
	Hand-wing area (×10 ⁻³ m ²)	-	-	-	-	-	25.42 ± 1.99
	Arm-wing area (×10 ⁻³ m ²)	-	-	-	-	-	58.56 ± 5.73
	Tip length ratio	-	-	-	-	-	0.68 ± 0.04
	Tip area ratio	-	-	-	-	-	0.44 ± 0.05
	Tip shape index	-	-	-	-	-	1.57 ± 0.15

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