



**Trap-nesting *Ancistrocerus sikhimensis* (Hymenoptera: Eumenidae) IN NEPAL: nest structure and associates (Hymenoptera: Chrysididae; Acarina: Saprogllyphidae)**

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TRAP-NESTING *ANCISTROCERUS SIKHIMENSIS* (HYMENOPTERA: EUMENIDAE) IN NEPAL: NEST STRUCTURE AND ASSOCIATES (HYMENOPTERA: CHRYSIDIDAE; ACARINA: SAPROGLYPHIDAE)

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ABSTRACT

The contents of 21 trap-nests located in Sagarmatha National Park, Nepal, in 2002 and 2003 revealed interesting aspects of the biology of *Ancistrocerus sikhimensis* Bingham (Hymenoptera: Eumenidae). The nests included 2-7 brood cells separated by mud partitions. The dimensions of these structures seem to increase from the first cell to the last one constructed by the wasp. Females always used all the available space of the trap-nests, and the variability in the number of cells per nest essentially depended on their different dimensions. All the emerged adults were females, and we suspect that this species is bivoltine, with a highly shifted sex ratio between the two generations. In 2002, the parasite *Chrysis* sp. aff. *coelestina* Klug, recorded for the first time on this host, was responsible for a rate of parasitism per nest of 0%-100%, with an average of 41.65%. A second cuckoo wasp, *Chrysis violenta ultramonticola* Linsenmaier, emerged from one nest in 2003. Most *A. sikhimensis* females housed, mainly on the abdomen, hypopi of the mite *Vespacarus* sp., which is known to be involved in other wasp-mite associations. Unlike other mite-symbiotic eumenid wasps, *A. sikhimensis* does not present an acarinarium on its body to house the mites.

Key Words: trap-nest, solitary wasp, parasitism, mutualism, Himalaya, Hymenoptera, Eumenidae, *Ancistrocerus sikhimensis*

RESUMEN

El estudio de los contenidos de 21 nidos trampa, localizados en el Parque Nacional de Sagarmatha, Nepal, ha dado a conocer aspectos interesantes de la biología de *Ancistrocerus sikhimensis* Bingham (Hymenoptera: Eumenidae). Los nidos contenían entre 2 y 7 celdas de cría, separadas por tabiques de barro, cuyas dimensiones—tanto de las celdas como de los tabiques—aumentaban según iban siendo construidas. En todos los casos las hembras construyeron celdas en todo el espacio disponible del nido, condicionando las dimensiones de éste el número de celdas por nido. Solo hembras emergieron de los nidos trampa, lo cual nos lleva a sospechar que esta es una especie bivoltina, con un sex-ratio diferente entre las dos generaciones. En el año 2002 el parásito *Chrysis* sp. aff. *coelestina* Klug, colectado por primera vez de este hospedador, fue el responsable de un alto parasitismo ( $\bar{x}$  (por nido) = 41.65%). En el año 2003 otro crisídido, *Chrysis violenta ultramonticola* Linsenmaier, emergió de un nido. Muchas hembras de *A. sikhimensis* albergaban, principalmente en el gáster, ácaros del género *Vespacarus* en el estado de hypopus, revelando una asociación entre estos artrópodos. A diferencia de otros euménidos simbiotes con ácaros, *A. sikhimensis* no presenta un acarinarium donde albergar a los ácaros.

Translation provided by the authors.

Most vespid wasps of the family Eumenidae nest individually. Females provision their brood with paralyzed larvae of Lepidoptera or Coleoptera, and no overlapping of generations exist (Iwata 1976; Krombein 1978; Bohart et al. 1982; Cowan 1991). They are mass-provisioners and prey are rapidly brought to the nest after the oviposition. Nests are built with materials such as mud or

chewed leaves, dug into the soil, or built in pre-existing cavities (Iwata 1976), and include several cells. When the wasp has collected enough food for one larva, she seals the brood cell and starts to work on a new one (Krombein 1967; Cowan 1991).

Females of the widely distributed genus *Ancistrocerus* Wesmael nest in pre-existing cavities, modifying them with mud (Berland 1928; Nielsen

1932; Blüthgen 1943, 1961; Bonelli 1969; Richards 1980), however *Ancistrocerus oiventris* Wesmael typically builds aerial mud nests (Berland 1928). The cavity adopted by the wasp may be a tube in the hollowed pith of a twig, an old insect bore-hole in rotten wood, or many other kinds of holes (Blüthgen 1943; Kurzenko 1981). Most species separate the cells with simple mud cell partitions, but in a few species, such as *Ancistrocerus antilope* Panzer, *A. parietinus* L., and *A. ichneumonideus* Ratzeburg, cells (located in the cavity) are entirely made of mud (Blüthgen 1943). This opportunistic nesting habit has allowed researchers to use trap-nests in order to study many aspects of the biology of these wasps (Krombein 1967; Jacob-Remacle 1976; Wearing & Harris 1999). *Ancistrocerus* females provision their nests with caterpillars of several families of Lepidoptera (Iwata 1976; Harris 1994). Some species show considerable seasonal variations in the sex-ratio (typically male-biased in winter and female-biased in summer) (Fye 1965; Longair 1981).

This paper offers basic information about the nest structure and the brood sex ratio of *Ancistrocerus sikhimensis* Bingham. Data come from a study carried out by placing a number of wood trap-nests in Sagarmatha National Park, Nepal. We also report data on three organisms associated with *A. sikhimensis* nests, two cuckoo wasps (Chrysididae), and a saproglyphid mite. Cuckoo wasps are very common parasites of eumenids (Alfken 1914; Nielsen 1932; Bonelli 1969). Associations between saproglyphid mites and solitary wasps are known for species belonging to different families, but in particular for Eumenidae (Cooreman & Crèvecoeur 1948; Krombein 1961, 1967; Mostafa 1970; Pekkarinen & Huldén 1991). This mutualistic relationship is complex and often includes venereal transmission of the mites through wasp genital chambers (Cooper 1955), or sometimes alternating parasitic and saprophagous phases of the mite on the host body (Okabe & Makino 2003).

## MATERIALS AND METHODS

### Study Area

The study was carried out in Sagarmatha National Park (27°45'-28°07'N, 86°28'-87°07'E; Solu-Khumbu district, Nepal). The average temperatures, measured during the study periods, were 12.9°C in 2002 and 13.4°C in 2003. In the study area, precipitation is concentrated in the monsoon season, lasting from the end of May to the end of September. In 2002 rain occurred, mostly in June, during 32% of the whole observation period (43 days) and in 2003 it rained every day (100%, 22 days).

### Trap-nests

Trap-nests of different sizes were made using wood of *Abies alba* (according to Krombein, 1967). The blocks (2 × 2 cm square section) were perforated to provide a suitable space where wasps could establish their nests. Seven different hole sizes were provided, with diameter ranging from 3 to 10 mm and tunnel length from 55 to 90 mm. After removal, the trap nest holes closed with mud were protected with a net or an adhesive tape, to avoid flights from each trap.

The trap-nests were later reopened by sawing along the longitudinal side and the collected material was preserved in 70% ethanol for determination. Head width of all specimens of *A. sikhimensis* and its chrysidid parasite collected in 2002 were measured to the nearest 0.1 mm.

### Collecting Sites

Seventy-one artificial nests were placed in 2002 and forty-six in 2003. In 2002 the nests, divided into 9 groups, were placed at two different sites from May 9th to June 20th and reopened on September 10th. Site 1 was a garden in Namche Bazar, 3450 m, on a south-facing slope. Site 2 was an open area outside of Kangyuma, 3700 m, on a north-facing slope. Two groups were placed at site 1, composed of 9 (1a) and 8 (1b) blocks. Block 1a was placed at 1 m<sup>x</sup> under the roof of a house, while block 1b was placed at 2 m<sup>x</sup> between stones in a wall in the proximity of a garden in bloom. At site 2, there were seven groups (2a-g). Block 2a was on a *Rhododendron arboreum* tree in bloom, and blocks 2b-c were in a stone wall in an open area near a wood of birches (*Betula utilis*) and rhododendron trees. Blocks 2d-g were under the roof of an isolated house. Block 2a was set at 1.5 m high, 2b-c at 1 m, and blocks 2d-g at 2 m above the ground. The nests that were not covered by roofs were protected from the rain with transparent plastic sheets.

In 2003, nests were placed (June 22nd) only at site 2, in the same location of groups d-g of the previous year, i.e., under the roof of a house, 2 m above the ground. On July 14th nests were removed and reopened the following spring (2 April 2004).

## RESULTS

### Nest Structure

In 2002, seven holes from site 2 were colonized (Table 1). We found two nesting species: *A. sikhimensis* and *Ancistrocerus* sp. (gr. *parietinus*) (Hymenoptera: Eumenidae). Six out of 7 nests were colonized by *A. sikhimensis*. Since they were all closed by a final plug of mud, they should be considered as completed nests. The number of cells

TABLE 1. NEST STRUCTURE, BROOD CELL CONTENTS, AND REPRODUCTIVE SUCCESS OF COLONIZED NESTS AT KANGYUMA IN 2002.

Nest	Ø hole (mm)	Tunnel length (mm)	N cells	N ♀♀/nest	N parasites	Rate of parasitism by <i>Chrysis</i> sp. aff. <i>Coelestina</i>
894	4	68	2	1*	1	50.0
829	10	90	5	0	2	100.0
879	6	80	4	4	0	0.0
762	6	80	7	7	0	0.0
750	6	80	6	4	2	33.3
844	6	80	3	1	2**	66.6
775	6	80	1	0	1	100.0

\*Larva (undetermined sex); \*\*1 cocoon. Nest 775 was colonized by *Ancistrocerus* sp., the other nests by *A. sikhimensis*. Determination was based upon new born emergence, but female owners of nests 894 and 775 were captured in the field.

per nest was very variable ( $\bar{x} = 4.14 \pm 1.87$ ; range = 2-7), but this value does not seem to be related to the length of the cavity (Table 1). Apart from the vestibular cells (*sensu* Krombein, 1967), in all the other cells we found a wasp or a parasite at the stage of adult, pupa, or larva. All the eumenid individuals collected were females ( $n = 16$ ), such that for each nest ( $n = 4$ ), the sex-ratio was 0 ♂♂ : 1 ♀♀ (Table 1). Head width of females ranged from 2.55 mm to 3.00 mm ( $\bar{x} = 2.76 \pm 0.14$ ;  $n = 16$ ). In two nests no vestibular cells were observed. No empty cells (*sensu* Krombein 1967) were found. The 4 (out of 5) empty cells found in nest 829 were probably used by the females as brood cells, but some eggs or the larvae at very immature stages were already dead from unknown causes. One additional nest (775) was colonized by another species of *Ancistrocerus*.

In all trap-nests the wasps used all the available space and the differences in the number of cells, for nests with equal burrow length, depended on the variable dimensions of the cells and/or the cell mud partitions. On average, both the cell lengths and the cell partition thicknesses were correlated with their order of construction (Table 2) (Spearman rank correlation;  $r_{\text{cells}} = 0.92$ ,  $r_{\text{cell partitions}} = 0.98$ ;  $n = 8$ ;  $P < 0.05$ ): vestibular cells and closing plugs were on average larger than the subsequent cell/cell partition, and the first cell/cell partitions (built by the wasp at the bottom of the nest) were the smallest ones.

In 2003, 14 active nests of *A. sikhimensis* were observed in the field. Only two of them were closed by the wasps, and they contained only larvae. In 8 nests we found adults of *A. sikhimensis*, sometimes associated with larvae. All the adults were females. In 6 other nests (included the two sealed ones) we found only dead larvae. In 4 nests we found also dry Lepidoptera larvae. In 2003, the observation of the activity of *A. sikhimensis* over six days, for a total of 10 h, gave the following results: (a) the wasps left the nest without any orientation flight (100%;  $n = 36$ ); (b) a collected prey was a larva of Lepidoptera (Glyphipterigidae); (c) the duration of provisioning flights ( $\bar{x} = 486 \text{ sec} \pm 524$ ) was not different from that of non-provisioning ones ( $\bar{x} = 704 \text{ sec} \pm 729$ ; Mann-Whitney Test;  $n_{1_{\text{pf}}} = 8$ ;  $n_{2_{\text{npf}}} = 20$ ; n.s.); (d) the time spent in the nest after a provisioning flight ( $\bar{x} = 347 \text{ sec} \pm 880$ ) was not different from that spent inside the nest after a non-provisioning flight ( $\bar{x} = 295 \text{ sec} \pm 405$ ; Mann-Whitney Test;  $n_{1_{\text{pf}}} = 11$ ;  $n_{2_{\text{npf}}} = 21$ ; n.s.).

#### Associates

Nest associates include Hymenoptera Chrysididae, and Acarina Saprogllyphidae. Cuckoo wasps belong to two species, *Chrysis* sp. aff. *coelestina* Klug (found in 2002) and *Chrysis violenta ultramonticola* Linsenmaier (3 specimens found in 2003 in one nest containing even one specimen of *A. sikhimensis*). The rate of parasitism due to

TABLE 2. LENGTH (MM) OF CELLS AND CELL PARTITIONS IN *A. SIKHIMENSIS* NESTS (2002).

	Cp	Vc	7°P	7°C	6°P	6°C	5°P	5°C	4°P	4°C	3°P	3°C	2°P	2°C	1°P	1°C
$\bar{x}$	2.8	26.0	2.3	15.7	2.0	12.5	2.0	10.4	1.5	9.0	1.3	10.0	1.0	10.0	1	8
SD	1.3	14.5	1.5	7.1	0.9	4.9	0.7	3.2	0.6	2.9	0.6	2.0	0.0	0.0	—	—
$n$	5	5	4	6	6	6	5	5	4	4	3	3	2	2	1	1
Range	1-4	15-24	1-4	6-26	1-3	5-19	1-3	6-15	1-2	5-12	1-2	8-12	—	—	—	—

Cp = Closing plug; Vc = Vestibular cell; P = cell partition; C = brood cell.

*Chrysis* sp. aff. *coelestina*, calculated for *A. sikhimensis*, ranged from 0% to 100%, and in general was high ( $\bar{x} = 41.65\%$ ;  $n = 6$ ) (Table 1). All specimens were females. Head width ranged from 2.00 mm to 2.65 mm ( $\bar{x} = 2.35 \pm 0.20$ ;  $n = 7$ ). On 15 out of 17 *A. sikhimensis* females collected from the 2002 nests we found individuals of the mite *Vespacarus* sp. (Acarina: Saprogllyphidae). The mites were located mainly on the dorsal side of the gaster of the wasps, between tergites I and II. Six individuals had a few mites on other parts of the body (eyes, wings, legs, neck, and thorax). All the mites were at the hypopi stage, the typical resting stage that these arachnids have evolved to face adverse conditions and as an efficient dispersal phase (Walter & Proctor 1999). The number of mites per wasp varied from 2 to 97, but these values should be considered underestimated because of the difficulty involved in counting all the specimens deeper in the abdominal tergites. We did not observe mites on the females emerging from the 2003 nests.

## DISCUSSION

### Nest Structure

As many other *Ancistrocerus* species, including the closely related *Ancistrocerus parietum* L. (Nielsen 1932; Krombein 1967), *A. sikhimensis* nests in pre-existing cavities. Although we did not find empty cells (*sensu* Krombein 1967), they have been reported in some species of *Ancistrocerus*, and they are probably constructed to better defend the brood from parasites (e.g. Krombein 1967). A shifted sex-ratio, female-biased in summer, has been recorded for other species of *Ancistrocerus*, probably to face the mortality due to cold winter climatic conditions, as assumed by other authors (Fye 1965; Longair 1981). In fact we do not know the influence of monsoon in sex allocation in this wasp. Moreover, considering a partial bivoltinism model (Seger 1983), we could expect that males live longer than females (males mate with females of their own and next generation), resulting in a female-bias. More data should be obtained to clarify sex-ratio dynamics in this species.

### Associates

Bonelli (1969) recorded *Chrysis ruddii* Shuckard for *A. oiventris*; and Alfken (1914), Van Lith (1953), Nielsen (1932) and Micheli (1930) recorded *Chrysis ignita* L. for *A. parietinus*, *A. antilope*, *Ancistrocerus nigricornis* Curtis, and *A. oiventris*. *Chrysis coeruleans* Fabricius and *Chrysis nitidula* Fabricius were recorded as parasites of *A. antilope* and *Ancistrocerus catskill catskill* (Saussure) (Cooper 1953; Hobbs et al. 1961; Medler 1964; Fye 1965; Krombein 1967). *Chrysis inflata* Aaron was found as a parasite of *Ancistro-*

*cerus durangoensis* Cameron and *Ancistrocerus tuberculiceps tuberculiceps* (Saussure) (Krombein 1967). *Chrysis* sp. aff. *coelestina* and *Chrysis violenta ultramonticola* are the first parasites recorded for *A. sikhimensis*.

Mutualistic associations between saprogllyphid mites and solitary wasps are known for different species: e.g., *Vidia concellaria* Cooreman and *Cerceris arenaria* L. (Crabronidae), *Vidia cooremani* Thomas and *Ectemnius* sp. (Crabronidae), several *Vidia* Oudemans, *Macroharpa* Mostafa, *Zethacarus* Mostafa, *Calvolia* Oudemans species and *Zethus* spp. (Eumenidae) (Cooreman & Crèvecoeur 1948; Baker 1964; Mostafa 1970). Okabe & Makino (2003) reported an association between *Kurosaia jiju* Okabe & O'Conner and *Anterhynchium flavomarginatum mikado* (Smith), where mites display an alternation of parasitic and saprophagous phases during their life cycle on the host. In many cases the transmission of mites from one individual to another is known to be venereal, because hypopi enter the genital chamber of the female host during copulation of the wasps (Cooper 1955; Okabe & Makino 2003).

For the genus *Ancistrocerus*, associations are known between some species with *Kennethiella trisetosa* (Cooreman) and *Ensliniella trisetosa* Vitz. (Cooper 1955; Baker & Cunliffe 1960; Krombein 1961, 1967; Cowan 1984). The genus *Vespacarus* Baker, as far as we know, is associated only with *Ancistrocerus catskill catskill* and *Ancistrocerus tigris tigris* (Saussure) (now *Ancistrocerus adiabatus* (Saussure)) (Krombein 1967: *Vespacarus tigris* Baker and Cunliffe). Contrary to what we observed in our wasp-mite association, in most *Parancistrocerus* species the hypopi of *Vespacarus* are located in an acarinarium between tergites I and II of the abdomen (Krombein 1967). *Ancistrocerus adiabatus* does not have a true acarinarium and hypopi simply cluster in transversal series under some posterior abdominal tergites (Krombein 1967). This species seems to provide an intermediate step toward species with a true acarinarium. This structure evolved in some hymenopteran species that have mutualistic relationships with saprogllyphid mites (Bequaert 1918; Giordani Soika 1985), possibly to increase the number of host mites or, maybe, to keep them in a fixed position of the body. However, the maximum (underestimated) number of 97 mites that we have obtained from a single wasp is close to the maximum load of mites (118) found by Krombein (1967) in the acarinarium of *Stenodynerus (Parancistrocerus) f. fulvipes* (Saussure). Clusters of *Kennethiella trisetosa* have been found on different parts of the body of *A. antilope*, such as the right side of the propodeum, the thorax, or on the back of the propodeum (Cooper 1955; Cowan 1984). Mites have been also found exclusively on the ventral surface of the thoracic segments of the host, although rarely on the head or the lateral

sides of the thorax (Okabe & Makino 2003). This would confirm the notion that different species of mite choose a specific part of the host body. On the other hand it seems that acarinarium of different wasp species, whenever they exist as in *Parancistrocerus*, are slightly differently shaped, possibly related to the specific mites they must house (Krombein, 1967). We collected specimens of *Vespacarus* sp. on females, but most studies have reported the presence of mites typically only on males (Cooper 1955; Krombein 1961; Pekkarinen & Huldén 1991). It has been proposed (Cooper 1955) that hypopi may be unable to infest the females' bodies because the female larvae eat the adult mites, while the male larvae allow the mites to live on them. Our observation demonstrate that this is not the only possible conclusion, and that probably other factors affect the survival of the mites in the wasp's nest cells of both sexes. In any case, owing to the absence of *A. sikhimensis* males in our nests, we do not know the extent of their infestation by part of *Vespacarus*.

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