1	Invisible but Identifiable: p-Chips as a Reliable Marking Method for Amazonian Bats
2	Juan J. Pellón <sup>1,2*</sup> , Naija Cuzmar <sup>1</sup> , Jorge L. Mendoza <sup>1,3</sup> , Cristian Tirapelle <sup>1</sup> , Nick Fluker <sup>1</sup> , Gideon
3	Erkenswick <sup>1</sup> , Mrinalini Watsa <sup>4</sup>
4	1. Field Projects International, Escondido, California, USA
5	2. Centro de Investigación Biodiversidad Sostenible (BioS), Lima, Peru
6	3. Samay Conservación, Lima, Peru
7	4. San Diego Zoo Wildlife Alliance, Escondido, California, USA
8	*Corresponding author: Juan J. Pellón (jpellon@ecologia.unam.mx)
9	Abstract
10	Marking techniques are essential for studying bat ecology and informing conservation efforts, yet
11	many existing methods present challenges related to size, tag detectability, and long-term retention.
12	P-Chips, ultra-miniaturized transponders detectable via red laser light, offer a promising alternative
13	to traditional banding or passive integrated transponer (PIT) tags. While their use has been
14	successfully demonstrated in captive bats, their effectiveness in free-ranging populations remains
15	largely untested. To evaluate the field applicability of p-Chips, we conducted a two-year field study
16	in the Peruvian Amazon, tagging 121 individuals across 21 species. We documented 23 recaptures,
17	with all p-Chips remaining functional over both short term (< 1 month) and long term (~ 1 year)
18	periods. Notably, no adverse effects such as scarring or tissue damage were observed. Red LED
19	illumination facilitated rapid tag detection, reducing handling time. Recaptured bats revealed high
20	site fidelity in most individuals, although some traveled over 1 km. These findings support the use of
21	p-Chips as a viable, detectable, minimally invasive, and cost-effective alternative to PIT tags,
22	particularly for small-bodied species. We recommend further research to optimize P-Chip technology

for broader application in wildlife tracking and conservation. 23

Keywords: Chiroptera, life tag, mark-recapture, Phyllostomidae, technology, wild bats 24

#### 25 Introduction

26 Individual identification of bats is critical for applied conservation research programs on population dynamics, aging, health and mortality (e.g. van Harten et al. 2022, Humphrey and Oli 2015, Jin et al. 27 28 2012, O'Shea et al. 2010, O'Shea et al. 2004, Cheng and Lee 2002). Researchers have historically 29 employed a variety of methods to individually mark bats for long-term monitoring (Kunz and Weise 30 2009). However, choosing the most effective marking technique remains a challenge, as available techniques vary in terms of cost, durability, practicality, and their impacts on animal health and 31 32 behavior (Reynolds et al. 2025, Lobato-Bailón et al. 2023, Markotter et al. 2023, Mellado et al. 2022, Kunz and Weise 2009). Effectiveness may also be species-dependent, necessitating the use of 33 multiple complimentary approaches (Kunz and Weise 2009, Bonaccorso et al. 1976). 34

Historically, forearm bands have been widely used due to their relatively low cost and ease of application (Kunz and Weise 2009). However, concerns over injuries, increased mortality, and potential interference with foraging activities in a range of species (Lobato-Bailón et al. 2023) have prompted researchers to explore alternatives (Seheult et al. 2024, Markotter et al. 2023, Kirkpatrick et al. 2019, Kunz and Weise 2009, Sherwin et al. 2002, Barnard 1989).

Passive integrated transponder (PIT) tags, a type of radio frequency identification (RFID) marker, have been frequently employed to permanently mark bats over the last few decades (Fontaine et al. 2024, Escobar et al. 2022, Locatelli et al. 2019, Britzke et al. 2014, Rigby et al. 2012, Ellison et al. 2007, Neubaum et al. 2005, Kerth and Reckardt 2003, Schooley et al. 1993, Barnard 1989). These subcutaneous tags encode a unique identification number that is readable by radio-based scanners, which can even be modified to automatically detect bats at roost entrances (Rivera-Villanueva et al. 2024, Adams and Ammerman 2015, Britzke et al. 2014).

Although evidence suggests that PIT tags do not negatively affect bats' body mass, body condition,
or reproductive success (van Harten et al. 2019, Locatelli et al. 2019, Rigby et al. 2012, Neubaum et

49 al. 2005), they have some limitations. Their application typically requires a large needle (12-gauge), 50 which may be invasive for smaller species. Tags are not externally visible, requiring handlers to 51 palpate the skin to locate them. They can migrate or even be occasionally expelled from the body, 52 leading to detection difficulties or data loss (van Harten et al. 2021; Rigby et al. 2012, Kunz and Weise 2009, Barnard 1989). Finally, they are cost-prohibitive at large scales, ranging between 5 and 53 54 10 USD per tag (Scheult et al. 2024); however, these prices vary depending on the vendor and the 55 quantity. Generally, however, PIT tags are preferable to forearm bands due to their higher retention 56 rates (van Harten et al. 2021; Ellison et al. 2007); however, the concerns over cost, detectability, 57 potential safety issues for very small bats (< 30 mm; < 5 g), and tag loss in some studies (e.g. Rigby et al. 2012) warrant investigation into alternative technologies. 58

P-Chips (p-Chip Corp., Chicago, Illinois) are ultra-miniaturized semiconductor transponders (500  $\times$ 500  $\mu$ m) that emit a unique ID when exposed to a red laser light (Pharmaseq 2012). Since the laser tip must be in close proximity to the tag for successful detection (< 1 cm), the tag is injected subcutaneously in an area with thin, translucent, and almost hairless skin via a narrow, 21 gauge needle, making them a promising alternative for marking even the smallest bat species in a more noninvasive way (Seheult et al. 2024, Ngamprasertwong et al. 2022, Gruda et al. 2010). P-Chips (1-2 USD per unit) can be also five to ten-fold less expensive than PIT-tags (Seheult et al. 2024).

Seheult et al. (2024) tested p-Chips in 30 captive *Eptesicus fuscus* (forearm length: 40 – 48 mm; weight: 11.6 – 30.8 g), inserting them in the skin of the wings and tibia. They found that the tags remained functional for over a year (464 days after tagging) while requiring minimal handling due to rapid detection by the scanner. However, they also noted that visibility decreased over time, which may complicate recapture efforts. This issue could pose a significant challenge in free-ranging bats, where uncertainty about previous tagging might lead to excessive handling in an effort to locate a potentially nonexistent tag. Given these challenges, Seheult et al. (2024) recommended testing them in more species and noncaptive conditions. In this study, we share results from using p-Chips in free-ranging Amazonian bats,
assessing their application, detectability, and retention across species.

76 Methodology

77 This study was conducted at the Estación Biológica Los Amigos (EBLA), located in the southeastern 78 Peruvian Amazon, at the confluence of the Los Amigos and Madre de Dios Rivers (12°30'-12°36'S,  $70^{\circ}02'-70^{\circ}09'W$ ). The region primarily consists of high and low terra firme forests, flooded palm 79 80 forests, and meandering river floodplain forests (MINAM 2015). According to "Servicio Nacional de 81 Meteorología e Hidrología del Perú" (SENAMHI), in Puerto Maldonado (~ 50 km away and the 82 nearest site), temperature ranges from 16.6°C to 32.2°C and monthly precipitation varies from 58 to 83 299 mm. At this site, an annual mark-recapture program for vertebrates has been ongoing since 2018, under which umbrella we were able to test this method for the individual identification of bats. 84

85 In 2023 and 2024, we conducted an annual bat mist netting program, using  $6 \times 3$  m and  $12 \times 3$  m mist 86 nets at accessible sites along the trail system at the field station (Watsa et al. 2023; Figure 1). Bats 87 were identified taxonomically using the dichotomous keys from López-Baucells et al. (2016) and 88 Díaz et al. (2021); and aged based on epiphyseal ossification (Brunet-Rossinni and Wilkinson 2009). 89 Each sampling site was typically sampled for four to five nights before moving to a new location. 90 Due to the limited availability of p-Chips, we prioritized tagging as many bats as possible within 4 91 specific sites to maximize the probability of future recaptures, rather than distributing tagged 92 individuals across all available sites for sampling in the station. The only exception was a Vampyrum 93 spectrum, which was tagged using a p-Chip we had reserved for exceptional captures of rare species 94 after exhausting our supply in the target sites. Our data on the recapture of p-Chip tagged bats includes 95 all surveyed sites, even those outside of active tagging periods. Each mist net's location at a sampling 96 site was georeferenced to measure distances between recapture events. We assessed tag functionality 97 within and across years by recording the distance and time between encounters of recaptured98 individuals.

99 P-Chips (p-Chip Corp.) are available in either preloaded or loose formats; in the latter case, they can 100 be manually loaded into injectors, which can be sterilized between uses or discarded. It should be 101 noted that the company is apparently not currently engaged in commercial sales of chips (P-Chip 102 Corp. personal communication). Researchers interested in using p-chips should therefore contact the 103 company directly to purchase them and adapt other needles for injection. The p-Chips were injected 104 using pre-loaded 21-gauge needles into the right forearm of each animal, approximately at the middle 105 (Figure 2, Video S1). Individual tag numbers were checked using the handheld reader (model WA-106 6000) connected to a Windows 10 laptop or tablet via USB. As per Seheult et al. (2024), we ensured 107 that each p-Chip was inserted into a disinfected injection site without flipping its orientation to 108 maintain detectability. During preliminary tests, we identified instances where some p-Chips were 109 unreadable or pre-loaded in a flipped orientation. For this reason, we checked each one before 110 injection by slightly exposing the p-Chip with the plunger of the needle to verify its readability and 111 orientation before implanting it. Additionally, the mark-recapture program involved taking fur for 112 toxicology analyses, and a wing punch for DNA barcoding; both of these took place at specific 113 anatomical sites and thus had the additional effect of serving as short-term marks.

This study was conducted under the permission RDG-000116-2021-DGGSPFFS (Servicio Nacional Forestal y de Fauna Silvestre, SERFOR), following the guidelines of the American Society of Mammalogists (Sikes et al. 2016) and with IACUC approval from Washington University in St. Louis and the San Diego Zoo Wildlife Alliance. For the full handling protocol, please see Watsa et al. (2023).

119 Results

120 Bats were sampled and tagged during two field seasons, each from June - August in 2023 and 2024 121 (Table 1). In the first season, p-Chips were placed on 24 bats, across 8 species. In the second season, 122 p-Chips were placed on 97 additional bats, across 19 species. In total, we inserted tags in 21 species 123 across three families (Phyllostomidae, Emballonuridae and Vespertilionidae). The forearm length of 124 the smallest tagged individual was 29.7 mm (Mesophylla macconnelli), and the lighter one weighed 125 5.4 g (Myotis riparius). On the other hand, the largest and heavier individual was a Vampyrum 126 spectrum with a forearm of 108.1 mm and, although we were not able to weight it, the weight for this 127 species ranges from 126 to 190 g (Medellín 2019).

128 Over the entire study period, we recaptured 22 individual bats across 23 recapture instances with p-129 Chips (Table 2, Table S3). Three individuals tagged in 2023 were recaptured in 2024, all other 130 recaptures occurred during the same field season in either 2023 or 2024. Of those three, two took 131 place near their original capture site (< 300 m), and one was approximately 1 km from its prior 132 location. For the remaining within-season recaptures: twelve occurred at the original capture site 133 within five days of their release, at a distance of less than 400 m; four were recaptured a month later, 134 also within a 400 m distance; and, three individuals were recaptured at a separate site, at distances 135 greater than 1 km. The smallest recaptured individual had a forearm length of 35.5 mm and weight of 4 g (Carollia benkeithi), and the largest, 66.8 mm and 39.2 g (Phyllostomus elongatus). 136

For recaptured individuals, the site of the injection was undetectable without any apparent scarring
or irritation to the skin. Although the p-Chip itself is not visible in species with dark skin (e.g. *Phyllostomus hastatus*), it is clearly noticeable with the aid of a red backlight (Figure 2, Video S2).

### 140 Discussion

Previously, Seheult et al. (2024) had tested p-Chips in captive *E. fuscus*, while Ngamprasertwong et
al. (2022) used them to study roost fidelity in *Craseonycteris thonglongyai*, the smallest bat in the
world. Our results provide the first evidence of their functionality in free-ranging bats within an open

and highly diverse ecosystem such as the Amazon. We observed that p-Chips can be inserted and
successfully read in the forearm of 21 bat species. The short-term functionality of the tags (from 1
day to 1 month) was confirmed in 19 individuals across five species, while long-term functionality
(around one year) was confirmed in three individuals from three species (Table 1).

148 All recaptured individuals that were supposed to have a working p-Chip, identified by other marks 149 (shaved hair or wing biopsy), retained the tag with full functionality. We demonstrate that inserting 150 p-Chips in the forearm is feasible and effective, supporting Scheult et al. (2024) conclusion that the 151 wing is the best location, even though we used forearm and not metacarpals for placement. By 152 implementing pre-injection verification, we did not observe any flipped p-Chips in the pre-loaded 153 syringes, except possibly in some used during the initial sessions before verification was applied. 154 However, we do not rule out the possibility of tags flipping over time, as noted by Seheult et al. 155 (2024). Additionally, we did not detect any notable scars or other negative effects of the p-Chip in 156 any recaptured bats, including those recaptured one year after tagging. These observations suggest 157 that tag loss in free-ranging bats may be low, as observed by Seheult et al. (2024) in captive bats. 158 Specifically, the protocol employed in this study (Watsa et al. 2023) is unlikely to cause morbidity or 159 mortality associated with application of p-Chips.

160 During the first sampling sessions, we had difficulty visually locating the p-Chip immediately after 161 injection or during recapture events. The issue was particularly pronounced for species with dark 162 skin, such as *Phyllostomus* spp. and *V. spectrum*, as the tag is not externally visible and could be 163 confused for natural markings and wounds. Eventually, we observed that by positioning a red LED 164 backlight beneath the wing, the p-Chip becomes clearly visible as a black, opaque square, even for 165 species with dark skin (Figure 2, Video S2). This technique allowed us to quickly confirm both the 166 presence and location of the p-Chip, reducing chip scanning time to approximately 5-10 seconds, and 167 resulting in a successful reading with a single approach of the detection laser. The implementation of this technique, coupled with increasing familiarity by our bat handling team, resulted in 100%successful deployment and scanning for placed p-Chips in 2024.

170 Although the number of recaptured bats may appear low, these recapture rates align with rates 171 recorded in the Amazon in other studies (e.g. Tavares et al. 2017, Ramos et al. 2010, Sampaio et al. 172 2003), and particularly, with previous findings at EBLA specifically (Bravo et al. 2008). 173 Comprehensive sampling in the Amazon is logistically challenging, as much of the habitat within a 174 given site is inaccessible. Even in areas with established trails, such as at the EBLA, it is difficult to 175 evaluate large areas simultaneously. Recapturing free-ranging bats here is even more complex, 176 considering that some species, such as Artibeus lituratus, can travel distances up to 113 km (Arnone 177 et al. 2016), and for most species, movement data is practically nonexistent. The majority of 178 recaptured bats in this study were found at short distances from their initial capture location, 179 indicating high fidelity to a specific roost or foraging area, at least in the short term (< 5 days). This 180 is particularly notable for two individuals (one C. benkeithi and one Artibeus obscurus) first captured 181 in 2023, as they were found foraging at the same site a year later. On the other hand, relatively long-182 distance movements were also detected, suggesting the opposite behavior in certain individuals. Our 183 findings highlight the value that could be gained from using p-Chips to conduct a large-scale mark-184 recapture program of all Amazonian bat species, which has been uncommon due to cost and feasibility 185 for some species. Future work can focus on unevenly distributed, high-resource sites that attract bats 186 from far distances, such as mammal clay licks, or major roost locations.

In addition to bats, p-Chips have been successfully used for marking and identification in animals of
various sizes, including fish (Spooner and Spurgeon 2024; Moore and Brewer 2021; Faggion et al.
2020), rodents (Clein et al. 2024; Warren et al. 2021; San Diego Zoo Wildlife Alliance 2016), crayfish
(Huber et al. 2023), salamanders (Moore et al. 2024), bees (Hamilton et al. 2019; Tenczar et al. 2014),
ants (Robinson et al. 2014, Robinson et al. 2009), and even ectoparasites (Folk et al. 2024). Although
most evidence comes from captive conditions, p-Chips have been shown to be effective identification

markers for wild fish (Spooner and Spurgeon 2024, Moore 2020), demonstrating no significant adverse effects and a tag retention rate of up to 94% after more than a year, even under harsh underwater conditions. Therefore, p-Chips are a suitable, considerably smaller alternative to PIT tags. Although p-Chips still require the recapture of marked individuals, unlike some PIT tags that are large enough to be detected by passive detector arrays, they represent a promising avenue for innovation for small-sized species due to their tiny size.

We fully agree with Seheult et al. (2024) that standardized protocols should be established to advance research on this technique. Our study contributes information on the long-term retention of p-Chips in free-ranging bats, the importance of proper insertion techniques, and the benefits of pre-injection confirmation and red light scanning to improve readability. These results suggest that p-Chips are an effective and minimally invasive method for longitudinal research on wild bats, offering a viable alternative to PIT tags, particularly for smaller species.

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362	<b>Table 1.</b> Number of individuals per species tagged with p-Chips during the 2023 and 2024 sampling
363	efforts at the Estación Biológica Los Amigos (Peru). Size categories were arbitrarily defined based
364	on the average forearm length (FA) of the captured individuals in this study. The weight of Vampyrum
365	spectrum could not be obtained. Measurements are just referential values rounded to integers.

Size category	Species	Weigth (g)	2023	2024
Small (FA: < 36 mm)	Carollia benkeithi	12	3	8
	Mesophylla macconnelli	9		1
	Micronycteris minuta	9		1
	Myotis riparius	6		3
	Rhinophylla pumilio	10	1	2
Medium (FA: 36 - 55 mm)	Carollia brevicauda	15	5	23
	Carollia perspiciallta	19	2	17
	Dermanura anderseni	12		1
	Dermanura gnoma	13		1
	Gardnerycteris crenulata	15		1
	Micronycteris hirsuta	15		1
	Saccopteryx bilineata	12		1
Large (FA: 55 - 75 mm)	Artibeus lituratus	72	1	
	Artibeus obscurus	38	4	12
	Artibeus planirostris	60	2	
	Lophostoma silvicola	35		8
	Phyllostomus elongatus	39		5
	Tonatia maresi	27		3
	Trachops cirrhosus	34		1
Very large (FA: > 75 mm)	Phyllostomus hastatus	101	6	7
	Vampyrum spectrum	-		1

368	<b>Table 2.</b> Bats marked with p-Chips and recaptured on a different day from their initial capture at the
369	Estación Biológica Los Amigos (Peru). The table includes the date of p-Chip insertion, as well as
370	the number of days and distance between the initial capture/marking and the recapture. Sex and age
371	(j, juvenile; a, adult) are provided for each individual. One specimen (Carollia brevicauda, first
372	row) was captured as a juvenile in 2023 and later recaptured as an adult in 2024. Tagging with p-
373	Chips was conducted only at sites 2, 3, 4, and 6. Detailed results are available in Supplementary
374	Table S3.

Bat individual	Date marking	Days to recapture	Distance traveled (m)	Marking site (S)	Recapture site (S)
<i>Carollia brevicauda</i> ♀j,a	21/07/2023	344	1014	<b>S</b> 3	S1
Artibeus obscurus ∂a	21/07/2023	334	234	<b>S</b> 3	<b>S</b> 3
<i>Artibeus obscurus</i> ∂j	21/07/2023	4	59	<b>S</b> 3	<b>S</b> 3
Carollia benkeithi $\stackrel{\bigcirc}{_+}a$	26/07/2023	334	22	S2	S2
<i>Carollia brevicauda</i> ∂a	26/07/2023	2	33	S2	S2
Phyllostomus elongatus 🖧 a	11/06/2024	32	194	S4	<b>S</b> 4
Carollia brevicauda $\begin{tabular}{l} a \\ \end{tabular}$ a	11/06/2024	4	45	<b>S</b> 4	<b>S</b> 4
<i>Carollia brevicauda</i> ∂a	11/06/2024	4	45	<b>S</b> 4	<b>S</b> 4
Carollia brevicauda $\begin{tabular}{l} a \\ \end{tabular}$ a	13/06/2024	2	0	<b>S</b> 4	<b>S</b> 4
<i>Carollia brevicauda</i> ∂a	13/06/2024	30	320	S4	S4
<i>Phyllostomus elongatus</i> <sup>Q</sup> <sub>+</sub> j	16/06/2024	1	140	<b>S</b> 4	<b>S</b> 4
Carollia brevicauda $\begin{tabular}{l} a \\ \end{tabular}$ a	17/06/2024	27	90	<b>S</b> 4	<b>S</b> 4
Carollia brevicauda $\begin{tabular}{l} a \\ \end{tabular}$ a	17/06/2024	26	16	S4	<b>S</b> 4
Carollia brevicauda $\begin{tabular}{l} a \\ \end{tabular}$ a	20/06/2024	17	1190	<b>S</b> 3	<b>S</b> 7
<i>Carollia brevicauda</i> ∂a	20/06/2024	3	160	<b>S</b> 3	<b>S</b> 3
Carollia perspicillata $\buildrel a$	20/06/2024	1	50	<b>S</b> 3	<b>S</b> 3
Artibeus obscurus ∂a	20/06/2024	17	1138	<b>S</b> 3	<b>S</b> 7
<i>Carollia brevicauda</i> ∂a	25/06/2024	2	80	S2	S2
Carollia benkeithi $\stackrel{\bigcirc}{_{+}}a$	25/06/2024	2	74	S2	S2
Carollia perspicillata ${}^{\bigcirc}_{+}a$	25/06/2024	3	435	S2	<b>S</b> 1
<i>Carollia benkeithi </i> ∂a	25/06/2024	2	160	S2	S2
<i>Carollia perspicillata</i> ∂a	25/06/2024	2	99	S2	S2

Figure 1. Bat capture sites at the Estación Biológica Los Amigos (Peru). Tagging with p-Chips was
performed in sites 2. 3, 4 and 6. All sites were evaluated for recaptures. The vegetation types follow
MINAM (2015).



Figure 2. P-Chips inserted in the middle of the forearm in different bat species at the Estación
Biológica Los Amigos (Peru), shown with and without red light. Arrows indicate the location of the
p-Chip. The scales are 5 mm. Additional photos are provided in Figure S2.



# **Supplementary material**

**Video S1.** Demonstration of p-chip placement and reading in a free-ranging *Phyllostomus elongatus* at the Estación Biológica Los Amigos (Peru). The images shown are just previews of the video. The video will be available at this link during the revision process: https://drive.google.com/file/d/1pIHgsqXfBpOb7tb8dfRu0pJMNg88YSR5/view.



**Figure S2.** P-Chips inserted in the middle of the forearm in additional individuals *Artibeus obscurus* and *Carollia benkeithi* at the Estación Biológica Los Amigos (Peru), shown with and without red light. Arrows indicate the location of the p-Chip. The scales are 5 mm.

## A. obscurus



## C. benkeithi

**Table S3.** Details on bats marked with p-Chips and recaptured on a different day from their initial capture at the Estación Biológica Los Amigos (Peru). The table shows the number of days (Dys) and distance between the initial capture/marking and the recapture in meters (Dist). Sex and age (j, juvenile; a, adult) are provided for each individual. One specimen (*Carollia brevicauda*, first row) was captured as a juvenile in 2023 and later recaptured as an adult in 2024. The first row in the timeline indicates the year (23, 2023; 24, 2024), while the second row represents the site (S). Green cells denote initial capture dates, while blue cells indicate recapture dates. Tagging with p-Chips was conducted only at sites 2, 3, 4, and 6.

			23	23	23	23	23	23	23	23	23	23	23	23	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Bat individual	Dve	Diet	<b>S</b> 3	<b>S</b> 3	S3	S2	S2	S2	S2	S4	S2	S2	<b>S</b> 4	S2	S6	S6	S6	S4	<b>S</b> 4	S4	<b>S</b> 4	<b>S</b> 4	S4	S4	S4	<b>S</b> 3	<b>S</b> 3	S3	<b>S</b> 3	S2	S2	<b>S</b> 1	S1	S1	S1	<b>S</b> 7	<b>S</b> 7	S7	<b>S</b> 7	S5	S4	<b>S</b> 4
	Dys	Dist	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Aug	Aug	Aug	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Jul
			21	24	25	26	27	28	29	30	31	1	2	3	7	8	9	10	11	12	13	14	16	17	18	20	21	23	24	25	27	28	30	1	2	4	5	8	10	12	14	15
<i>Carollia brevicauda</i> ♀j,a	344	1014																																								
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