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First case of a Great Spotted Woodpecker (*Dendrocopos major*) egg in a Cinereous Tit (*Parus cinereus*) nest

Jian Fang,¹ Xudong Li,² Wei Liang,³ Jiangping Yu,⁴ Haitao Wang,^{2,4} Guodong Yi,^{1*} and
Romain Lorrilliere⁵

ABSTRACT—In the breeding season of 2018, we found a much larger egg in one nest of Cinereous Tit (*Parus cinereus*) in Zuojia Nature Reserve, Jilin Province, China. The tit pair abandoned their nest soon after the alien egg hatched, which resulted in the death of the foreign chick. Based on DNA identification, this foreign chick was confirmed as a Great Spotted Woodpecker (*Dendrocopos major*), suggesting that the woodpecker laid or carried one of her own eggs into the tit nest. Limited cavity availability as the woodpecker lost its own nest might have contributed to this occurrence. Received 8 November 2019. Accepted 22 July 2021.

Key words: cavity-nesting birds, DNA identification, foreign chick.

大山雀 (*Parus cinereus*) 巢中发现一枚大斑啄木鸟 (*Dendrocopos major*) 卵的首例报道

摘要 (Chinese)—2018 年的繁殖季节, 我们在中国吉林省左家自然保护区内繁殖的大山雀 (*Parus cinereus*) 巢中, 发现了一枚异常大的卵。待这枚寄生卵出雏之后, 大山雀不久便弃巢并导致该寄生雏鸟的死亡。通过 DNA 鉴定该寄生雏鸟是大斑啄木鸟 (*Dendrocopos major*), 意味着大斑啄木鸟将自己的卵产于或者挪到了大山雀的巢内。推测可能是由于大斑啄木鸟失去了自己的巢, 而有限的巢资源导致了该案例的发生。

关键词: 寄生雏, 洞巢鸟, DNA 鉴定

¹
School of Life Sciences, Jilin Normal University,
Siping, China

²
Jilin Engineering Laboratory for Avian Ecology and
Conservation Genetics, School of Life Sciences, Northeast
Normal University, Changchun, China

³
Ministry of Education Key Laboratory for Ecology of
Tropical Islands, Key Laboratory of Tropical Animal and
Plant Ecology of Hainan Province, College of Life
Sciences, Hainan Normal University, Haikou, China

⁴
Jilin Key Laboratory of Animal Resource Conservation
and Utilization, Northeast Normal University,
Changchun, China

⁵
Centre d'Ecologie et des Sciences de la Conservation
(CESCO, UMR 7204), Museum national d'Histoire
naturelle, Centre National de la Recherche Scientifique,
Sorbonne Université, Paris, France

* Corresponding author: guodongyi@126.com

Birds that provide parental care increase significantly the survival probability of their offspring, but at the price of a high cost for reproduction. In consequence, they can only raise a limited number of offspring (Shen et al. 2011). For females, a way to reduce the parental care cost is to lay all or some of their eggs in another nest (Cichon 1996). This behavior, named brood parasitism, can be done in intraspecific or interspecific nests (Davies 2000, Gong et al. 2016).

For cavity-nesting birds, the entrance opening can help prevent interspecific brood parasites' access to nests (Grim et al. 2014, Liang et al. 2016, Stokke et al. 2018). Even with such protection, several cases of cavity-nesting birds laying eggs in the nest of another cavity-nester have been published (Wiebe 2000, Robinson et al. 2005). Because most studies used artificial nest boxes with small entrance holes and interspecific brood parasitism in natural cavities is difficult to detect, only a few cases of tit (*Parus* spp.) parasitism have been found in recent decades (e.g., Goertz 1977, Campbell et al. 2001, Grim et al. 2014, Liang et al. 2016).

Intraspecific nest parasitism, in turn, is much less known due to the difficulty to detect it, often requiring genetic analyses (Stevens 2013). However, such instances have already been reported in 234 species, mainly in precocial or colonial birds (many of them in the orders Anseriformes, Galliformes, and Charadriiformes) and less so among altricial species and cavity-nesting passerines such as *Troglodytes* and *Sturnus* (Kempenaers et al. 1995, Yom-Tov 2001). Here, we provide the first report of a Great Spotted Woodpecker (*Dendrocopos major*) egg in the nest of a Cinereous Tit (*Parus cinereus*, Vieillot, 1818, syn. *Parus major cinereus*, herein "tit") in a nest box.

Our study area is located at Zuoja Nature Reserve (4481°–4580°N, 12680°–12688°E) in Jilin Province, northeastern China, at elevations that ranged from 200 to 500 m. We monitored a Cinereous Tit population in nest boxes during the breeding seasons (Apr–May) from 2009 to 2018. The number of nest boxes (12.5312.5325 cm in size with an entrance diameter of 4.5 cm)



Figure 1. A Great Spotted Woodpecker egg in a Cinereous Tit nest. (a) 7 May 2018: one alien egg and 11 tit eggs; (b) 17 May 2018: the alien egg was the first to hatch.

distributed in our study area was kept at about 450 per year. We visited nest boxes at least once a week to assess the first egg date and clutch size (Yu et al. 2017). About 30% of the nest boxes were used by secondary cavity-nesting birds, including the Cinereous Tit, Yellow-rumped Flycatcher (*Ficedula zanthopygia*), Daurian Redstart (*Phoenicurus aureoreus*), and Eurasian Nuthatch (*Sitta europaea*). The Cinereous Tit is the dominant species in our study area; on average, they occupy 13.4% (between 8% and 26.4%, depending on the year) of all monitored nest boxes.

On 2 May 2018, we found 6 tit eggs in one of the nest boxes used by a pair of Cinereous Tits and deduced that the first egg date was 27 April. However, when we rechecked the nest box on 7 May, we found 1 much larger, solid white egg and 11 tit eggs (Fig. 1a). The female started incubating on 8 May, and the alien egg was the first to hatch on 17 May (Fig. 1b). Three days later, we found the alien chick dead in the nest, and none of the tit eggs had hatched (all tit eggs were cold). We removed the dead alien chick and left the tit eggs in this nest box to observe whether the parent birds would return to the nest. Unfortunately, we found the same set of cold tit eggs on 23 May—the tit pair seemed to have deserted the nest.

Because identifying this alien chick to species was difficult based on morphology alone, we used a DNA-based method. The liver and the leg muscle were dissected from the chick carcass, and we stored the tissues in ethanol at an absolute concentration, then stored them at 20 °C until the DNA was extracted. We separately extracted the DNA from the liver and the muscle tissue samples using the UNIQ-10 column animal genomic DNA isolation kit (SK1206, Sangon, Shanghai, China), and amplified target DNA segments using PCR (polymerase chain reaction). DNA segments from cytochrome b genes were amplified by using the PCR primers L14841 and H15149 (see details in Kocher et al. 1989; Table 1) and then we analyzed the similarity of DNA fragments from the 2 samples with the sequences in a database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). As a result, we found the alignments of this chick were 99.7% and 99.4% similar to mitochondrial DNA of the Great Spotted Woodpecker (GenBank accession no. KT350609), respectively.

The incubation period of Great Spotted Woodpecker eggs is 10–13 d (Smith 2005, FergusonLees et al. 2011, Smith and Smith 2019). The woodpecker egg hatched successfully in this situation because its incubation period (10 d) matched closely that of the tit (10–15 d for this population, JY, pers. obs.). The circumstances leading to this woodpecker egg ending up in a tit nest are less clear. Here, we speculate that a female Great Spotted Woodpecker laid or carried this egg

Table 1. Primer sequences and sources of cytochrome b used to identify a Great Spotted Woodpecker chick in a Cinereous Tit nest.

Name	Sequence
L 14841	5'-CCATCCAACATCTCAGCCATGATGAAA -3'
H 15149	5'-TGCAGCCCTCAGAATGATATTGTCTCA -3'

into the tit nest. Such behavior is poorly documented in Great Spotted Woodpeckers. Below we discuss 3 possible explanations.

A side-effect of cavity limitation or competition—Some woodpecker species are common nest usurpers (e.g., Rothenbach and Opio 2005, Kronland 2007). The forest type in our study area was a secondary forest about 45 years of age. Suitable nesting trees, such as Mongolian oaks (*Quercus mongolica*), Dahurian birches (*Betula dahurica*), and Manchurian linden (*Tilia mandshurica*) are numerous at our site (Deng and Zhang 2016) and nest sites are probably not limiting for woodpeckers. Moreover, no cases of the Great Spotted Woodpeckers occupying nest boxes have been found in our study area. We deduct that a failed attempt at nest usurpation by Great Spotted Woodpeckers is unlikely.

Intentional brood parasitism—Woodpeckers are not obligate brood parasites (Stevens 2013) and this particular species is not known to be an interspecific brood parasite. It is possible that the woodpecker made a mistake looking for another woodpecker’s cavity to parasitize its nest. This is also unlikely, because the nest materials used by Cinereous Tits (lots of green moss and several types of nest linings) and Great Spotted Woodpeckers (no nest lining) are quite different (e.g., Harnicarova and Adamik 2016). Therefore, we do not think that this Great Spotted Woodpecker mistakenly laid 1 egg in another species’ nest intending to parasitize the conspecific’s nest. Furthermore, while conspecific parasitism is known to

occur in some secondary cavity-nesters (birds that do not excavate the cavities themselves) driven by a lack of available cavities, this behavior is not known in primary cavity excavators such as woodpeckers (Eadie et al. 1998). Thus, no intraspecific brood parasitism in the Great Spotted Woodpecker has been reported to date (Michalek and Winkler 2001, Yom-Tov 2001) and so an intentional instance of brood parasitism is therefore an unlikely explanation.

Opportunistic and emergency brood parasitism—Several hypotheses attempt to explain opportunistic parasitism from non-obligate brood parasites. This behavior could be a strategy to address brood size constraints to increase offspring survival or reduce parental care costs and improve adult survival. Also, a female having lost her nest during egg-laying can recover part of the reproductive effort by laying the rest of her clutch as a nest parasite (Lyon and Eadie 2008). Hickman (1970) observed a Red-bellied Woodpecker (*Melanerpes carolinus*) carrying an egg out of its nest, probably due to the destruction or usurpation of its primary cavity. In our case, the Great Spotted Woodpecker female might have laid the egg herself in her own nest, but her cavity may have been destroyed during the egg-laying period, so she carried the egg to the tit's nest. Alternatively, the female woodpecker could have been ready to lay her egg, but due to nest loss had to find an alternative nest as soon as possible. If no other Great Spotted Woodpecker nest was available for conspecific brood parasitism, the egg had to be laid in another species' nest (Fowler Neal and Rolland 2015). Here, we suggest that the limited availability of cavities, such as the woodpecker losing its own, might have contributed to this occurrence.

Previous studies suggest that only hosts under pressure from brood parasitism have evolved antiparasite strategies (e.g., Rothstein and Robinson 1998, Davies and Welbergen 2008, Davies 2011). Host Cinereous Tits in our study area can reject foreign eggs (70% egg-rejection rate; see details in Liang et al. 2016). Here, however, tits abandoned their nest with an alien chick. Our report suggests that cavity-nesting birds have the ability to evolve resistance mechanisms against brood parasites (Liang et al. 2016), a strong evolutionary driver in this study system (Medina et al. 2020).

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