Ophiocordyceps neonutans sp. nov., a new neotropical species from O. nutans complex (Ophiocordycipitaceae, Ascomycota)

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Abstract

Ophiocordyceps nutans is an entomogenous fungus growing on true bugs (Hemiptera), which has a presumed worldwide distribution. During forays of entomogenous fungi in Brazil, specimens morphologically similar to O. nutans were collected from the Atlantic Forest and Cerrado domains of Neotropical region. Morphological comparisons, as well as molecular phylogenetic analyses using ITS, led us to conclude that the neotropical specimens represent a new species Ophiocordyceps neonutans. The Neotropical occurrence of this taxon and its taxonomic implications are re-evaluated here. We discuss O. nutans as a species complex with distinct geographic lineages and host specificity. In addition, Barcoding gap analysis suggests that the different lineages have a great genetic distance between them.

Key words: Cordyceps s.l., entomogenous fungi, Hemiptera, Pentatomidae, bugs

Introduction

Ophiocordyceps Petch (1931: 73) is the largest genus in Ophiocordycipitaceae (Hypocreales, Ascomycota), comprising more than 200 species (Sung et al. 2007, Spatafora et al. 2015, Shrestha et al. 2017a, Wijayawardene et al. 2017). The genus is characterized mainly by darkly pigmented, rough, pliant or wiry stroma that parasitizes larvae and adults of different arthropod orders (Sung et al. 2007, Wijayawardene et al. 2017).

Ophiocordyceps nutans (Pat.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007: 45) was described from Japan growing on an adult bug (Hemiptera) (Patouillard 1887), and is characterized by its yellow to reddish orange, apical, cylindrical to sometimes oval head, and by the black stipe (Kobayasi 1941, Sasaki et al. 2008). The species is mostly recorded for Asia, but it is also occasionally reported from Africa, South America and Oceania.

The first record of O. nutans in Brazil was presented from the Southern Atlantic Forest domain of the State of Paraná on pentatomid bugs (de Meijer 2006) and the only other citation of O. nutans in Neotropics was made by Kobayasi (1981) from Colombia, but neither description nor illustrations or host information was provided in either work. During numerous surveys of entomogenous fungi in Brazil, we collected several specimens morphologically identified as O. nutans; however, the occurrence of this taxon in Neotropics should be re-evaluated, mainly because some of our specimens indeed represent a new species based on molecular, ecological and morphological evidences.
Materials and Methods

Study area and hosts:—Surveys of entomogenous fungi were made between 2011 and 2014 in the Atlantic Forest domain of the States of Santa Catarina, Paraná and São Paulo (Ombrophilous forests of different conservation units), and in the Valley forest of Cerrado domain of the State of Mato Grosso of Brazil (Valley Forest of Chapada dos Guimarães National Park). According to the Köppen classification (Köppen & Geiger 1928), the Atlantic Forest domain presents well-distributed rains throughout the year and rigorous climate during the summer; the climate in Cerrado domain is characterized by being hot and humid, with rainy (October to March) and dry (April to September) seasons.

Specimens, morphological studies:—The specimens were dried at 40ºC, or in plastic bags with silica when they are quite fragile, and deposited at the FLOR herbarium, Universidade Federal de Santa Catarina. Colors were determined following Anonymous (1969). Free-hand longitudinal sections of the apical heads were mounted on lacto fuchsin to observe perithecial structures, asci, ascospores and part-spores. Ascospores were also observed after discharge, with cover slips placed under the fertile parts of fresh specimens in humid condition. The measurements were made (n=20) according to the method of Sasaki et al. (2008), using Olympus CX21 microscope.

Specimens from KW herbarium and personal collections of Dr. Sasaki (Sasaki et al. 2012) were studied for morphological comparisons. Herbaria acronyms follow Index Herbariorum according to Thiers B. (continuously updated).

DNA extraction and sequencing:—Extraction of total genomic DNA from dried stromata was performed according to Doyle et al. (1987) and Góes-Neto et al. (2005). Primers ITS1-R and ITS4-F (White et al. 1990) were used for amplification of nrITS region (ITS1-5.8S-ITS2), according to the cycle parameters described in Sasaki et al. (2012). Sequencing was performed with BigDye Terminator v.3.1 Cycle Sequencing Kit following manufacturer’s procedures at small-scale sequencing facility at Centro de Pesquisas René Rachou Fiocruz-MG (Brazil).

Phylogenetic analyses:—A total of 39 sequences representing six putative species of Group 2 in clade III of Cordyceps sensu lato (Stensrud et al. 2005), herein referred to as “sphecocephala” clade, were used to construct the matrix. Metarhizium taii Z.Q. Liang & A.Y. Liu (1991: 260) was included as an outgroup, based on previous studies, which showed it forming a sister-relationship with “sphecocephala clade” (Stensrud et al. 2005). All the sequences used in this study are listed in Table 1. Sequences generated in this study were assembled and manually corrected with Geneious v. 6.1.8 (Kearse et al. 2012), then automatically aligned with Mafft v.7 (Katoh & Standley 2013) under the Q-INS-I strategy, and then manually inspected and adjusted as necessary, searching for unreliably aligned positions, with MEGA 6 (Tamura et al. 2013). The final alignment as well as the resulting phylogenetic trees were deposited in TreeBASE (http://www.treebase.org/treebase/index.html) under S20271. The indels present in nrITS were recorded as binary characters following the simple indel coding method (SIC, Simmons & Ochoterena 2000) as implemented in the SeqState software (Müller 2005). Two distinct analyses were applied to the dataset: Maximum Likelihood (ML), as implemented in RAXML v.8.1.24 (Stamatakis 2014), available in the CIPRES science gateway (Müller et al. 2010); and Maximum Parsimony (MP), as implemented in the software PAUP* 4.0b10 (Swofford 2003). For the ML searches, the nrITS dataset was subdivided into three data partitions, ITS1, 5.8S and ITS2, plus the binary partition represented by the recoded indels. The best-fit model of nucleotide evolution for dataset was obtained according to Akaike Information Criterion, as implemented in the software jModelTest v.2.1.4 (Guindon & Gascuel 2003, Darriba et al. 2012).

For the ML approach, the analysis first involved 100 ML searches, each starting with one randomized stepwise addition parsimony tree under the GTRGAMMA model, with all the other parameters estimated by the software. Only the best-scoring ML tree from all the searches was maintained. To access the reliability of the nodes, multiparametric bootstrap (BS) replicates under the same model were computed, allowing the program to halt bootstrapping automatically through the autoMRE option. We provided an additional alignment partition file to force RAXML to estimate for independent parameters for each partition.

For the MP analyses, gaps were treated as missing data, and the most parsimonious trees (MPT) were sought with heuristic searches with 1000 random-addition-sequence replications, with the starting trees obtained through stepwise addition. The branch-swapping algorithm used was Tree Bisection-Reconnection (TBR). The reliability of the nodes was evaluated by 500 BS pseudoreplications, with the results used to obtain a 50% majority rule in the bootstrap consensus tree. Analysis parameters were: steepest descent not in effect and MULTREES effective.

For both analyses, a node was considered to be strongly supported if it showed a BS CHES with 1000 random-addition-sequence replications for each partition.

Barcode gap analysis:—The Barcode gap analysis was carried out according to Badotti et al. (2017), using sequences generated in this study and those of Sasaki et al. (2012), representing three different hypothetical species.
Sequences were aligned using MUSCLE (version 3.8.31) with default parameters (Edgar 2004), and distance matrices were generated using the uncorrected p-distance due to its simplicity and absence of any biological assumptions (Russo et al. 2012). A species was considered successfully identified if the minimum interspecific distance was larger than its maximum intraspecific distance (Hollingsworth et al. 2009). Custom Perl scripts were written to calculate the distance matrices. Jitter plots were generated using R (R Development Core Team 2016).

### TABLE 1. Specimens and voucher used in the phylogenetic analysis.

<table>
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<tr>
<th>Species</th>
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<th>Source of sequences</th>
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### Results

**Molecular phylogeny:**—New nrITS sequences from six Brazilian specimens were generated in this study. The nrITS dataset of all the 40 sequences used in this study (Table 1) represented seven species (including out-group). The final gapped nucleotide alignment was 619 bp, besides 129 recoded indels. In the mixed matrix (748 characters long), 306 characters were constant, 105 variable characters were parsimony-uninformative, while 337 were parsimony-informative. The models of evolution selected to each nucleotide partition were HKY+G to ITS1, TPM2+G to 5.8s and TPM2+G to ITS2. The trees generated by both phylogenetic searches (ML and MP) showed similar topologies.
Nine of the internal branches (ingroup) in ML and twelve in MP appear with significant support values. Only the topology from ML analysis is presented, while both BS values for ML and MP showed on the branches (Fig. 1). In our recovered phylogenetic hypothesis based on nrITS, the Brazilian specimens were more closely allied with other species in the "sphecocephala" clade than *O. nutans*. Two main clades were recovered. One of these clades grouped the two distinct lineages identified as *O. nutans*, both from Asia, as previously observed by Sasaki *et al.* (2012), with moderate significant support in MP (BS=83), and no significant support in ML (BS=59). One of those lineages is composed by the Type I that parasitizes Coreidae members (ML/BS = 72 and MP/BS= 100), and the other by the Type II that parasitizes members of three families Urostylidae, Acanthosomatidae and Pentatomidae (ML/BS = 60 and MP/BS= 100). The second main clade groups the other five species of the "sphecocephala" clade, and received moderate support in ML (BS=79) and maximum in MP (BS=100). Within this clade, the new species appeared as a sister to the remainder ones, with maximum support in both analyses. *Ophiocordyceps irangiensis* (Moureau) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007: 43) groups together with *O. sphecocephala* (Klotzsch ex Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007: 47) with no significant support, while *O. myrmecophila* (Ces.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007: 45) groups with *O. tricentri* (Yasuda) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007: 47) (Fig. 1).

**FIGURE 1.** Phylogenetic relationship among *Ophiocordyceps nutans*, *O. neonutans* and other *Ophiocordyceps* species based on rDNA internal transcribed spacer (ITS) sequences. Consensus tree (MV) obtained from the heuristic search is presented. The trees generated by both phylogenetic searches (BPP and ML) showed similar topologies.

**Barcode gap analysis:**—The Brazilian and the two Asian (Type I and Type II) clades were confirmed based on barcode gap analysis (Fig. 2). This analysis corroborated that the Brazilian specimens have a great genetic distance from Asian specimens (Type I and Type II) showing three distant and well-delimited groups.

For *O. nutans* complex, there is a clear and marked barcode gap between the two ranges of genetic variability datasets (inter and intraspecific). The intraspecific variation is low, *i.e.*, there is little variation in genetic distances between distinct specimens inside a same group; however, there is a high interspecific variation between the groups as evidenced by a barcode gap ranging from 5–17%.

In this paper, we introduce a new species mostly with support from molecular data. We follow the recommendation of Jeewon & Hyde (2016) for introducing a new taxon based on molecular data.
FIGURE 2. Barcode Gap analysis between *Ophiocordyceps nutans* and *O. neonutans* based on the intraspecific and interspecific distances based on ITS region. Each dot represents a pairwise comparison. All interspecific pairwise comparisons are plotted in the column “A” and all intraspecific ones are plotted in the column “B” of the axis X. Genetic distances are plotted in the axis Y.

**Taxonomy**

*Ophiocordyceps neonutans* R. Friedrich, B. Shrestha & Drechsler-Santos, sp. nov.  
(Figs. 3, 4)  
Mycobank:—MB819384  
Type:—BRAZIL. Santa Catarina: Florianópolis, Parque Estadual da Serra do Tabuleiro, Naufragados, 10 January 2014, *Friedrich KEL113* (holotype FLOR57336!).

**Diagnose:**— *Ophiocordyceps neonutans* is morphologically similar to *O. nutans*, however is more robust.  
**Etymology:**—Referring to the Neotropical locality of the new species and its morphological similarity to *O. nutans*.  
Stromata solitary, rarely two, simple or branched (32–170 × 1–2 mm). Stipe filiform, erect or somewhat curved, black (23–151 × 1 mm), becoming orange to orange reddish (47/48) towards the uppermost part of the stipe in immature condition and similar to head in color at maturity, pale yellow (9/8) when dry. Fertile head apical, well delimited...
(5–19 × 0.9–2 mm), cylindrical, oblong to fusiform, orange punctate with brown (12) ostioles of perithecium. Perithecium crowded, obliquely vertical in the head, completely immersed, piriform, always with a long curved neck (550–1200 × 130–360 mm). Ascii 8-spored, cylindrical, hyaline (220–900 × 3–8 µm), with a prominent cap. Ascospores parallel, smooth, filiform, almost as long as the asci; hyaline, multiseptate, easily fragmenting into 64 part-spores; part-spores cylindrical (6–15 × 1.2–3 µm), or slightly barrel-shaped. Mycelia inside insect body is slightly pink with a cork appearance. Spore mass is white.

**Host and distribution:**—On adult stinkbugs belonging to subfamilies Edessinae, Discocephalinae and Pentatominae of Pentatomidae (Hemiptera). Mature specimens were mostly collected from November to April. They were recorded for the Cerrado (State of Mato Grosso) and Atlantic forest (States of Santa Catarina, Paraná and São Paulo) domains of Brazil. Although the specimens were collected in different ecosystems, they were always found near rivers or in areas with high mean annual rainfall.

**Remarks:**—*Ophiocordyceps neonutans* is frequently collected as a single, black stroma, with a cylindrical orange head in hosts buried in the upper 1–2 cm of the leaf litter. The arrangement of ascospores within the asci is parallel for the entire length of the asci, indicating that the ascospores are approximately of the same length as asci. The anamorph could occur in the same stroma, right beneath the fertile head in the orange part of the stipe, or in another stroma (Fig. 3A, 3B). *Ophiocordyceps neonutans* is very similar to *O. nutans* from Asia (Type I and Type II); however, it slightly differs in stromata colour and shape, perithecia shape and width (Fig. 4), host species and geographic distribution. *Ophiocordyceps neonutans* is more similar in micromorphology to *O. nutans* Type I (Sasaki et al. 2012), but in macromorphology, it is more similar to *O nutans* Type II. It is worth noting that the hosts of *O. nutans* Type II are more similar as those of *O. neonutans* (Pentatomoida). *Ophiocordyceps neonutans* appears to be larger and more robust than the Asian specimens. Additionally, *O. neonutans* has a more crowded stroma with perithecium, the fertile orange region is cylindrical to fusoid, never ovoid, and parasitizes different subfamilies of Pentatomidae. *Ophiocordyceps neonutans* is also morphologically similar to *O. tricentri* that has been recorded only from Asia, parasitizing spittlebugs (Hemiptera), and with different color of stipe, shapes of perithecium and ascospores (Shrestha & Sung 2005, Shrestha 2011). *Ophiocordyceps neonutans* also has similar characteristics to few other species in the “sphecocephala” clade, however all species of that clade are parasites of different host species (Stensrud et al. 2005).


FIGURE 3. *Ophiocordyceps neonutans*: (A) a specimen in the field, (B) enlarged head with (B*) brown ostioles, (C) perithecia, (D) mature ascus with ascospores, (E) fragmented part spores. (scale bar: A, B = 15 mm, C, D = 300 μm, E = 15 μm).
FIGURE 4. *Ophiocordyceps neonutans* versus *O. nutans*: (A, B) Brazilian specimens of *O. neonutans*; (C) Japanese specimens of *Ophiocordyceps nutans* (scale bar = 15 mm).

**Discussion**

*Ophiocordyceps nutans* is traditionally recognized by its yellow, orange or red, apical, cylindrical to ovoid head, and a black, filamentous stipe growing on an adult bug, characterized by obliquely immersed perithecia, and easily
...fragmenting ascospores (Kobayasi 1941) (Fig. 4). *Ophiocordyceps neonutans* shares many of these characteristics with *O. nutans*; however, the fertile, apical region of *O. neonutans* is cylindrical to fusoid and the apical region possesses narrower and longer perithecia (Figs. 3, 4). The studied materials sometimes produced needle-like apex on the stipe, indicating that the specimens were immature, as mentioned by Sasaki et al. (2004). The perithecia of *O. neonutans* (550–1200 × 130–360 µm) were narrower than those of Japanese *O. nutans* (550–1170 × 190–560 µm) (Sasaki et al. 2008), but larger than those of Thai (550–800 × 130–300 µm) (Hywel-Jones 1995) or Nepalese *O. nutans* (800–900 × 300 µm) (Shrestha & Sung 2005) (Table 3). Macromorphically, stromata and the fertile regions of Japanese materials are thinner and delicate than those of *O. neonutans*, which are more robust and ridged (Fig. 3). According to Hywel-Jones (1995), the Thai specimens appear to have a wider fertile region than others (Table 2).

**TABLE 2.** Macromorphological comparison between *Ophiocordyceps nutans* and *O. neonutans*.

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<th>Fertile head (mm)</th>
<th>Host (mm)</th>
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**TABLE 3.** Micromorphological comparison between *Ophiocordyceps nutans* and *O. neonutans*.

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<tbody>
<tr>
<td><em>O. nutans</em> (Type II) - Japan Pentatomidae</td>
<td>610–1150 × 190–560</td>
<td>225–850 × 5–9</td>
<td>4–20 × 1.5–2</td>
</tr>
<tr>
<td><em>O. nutans</em> (Type II) - Japan Acanthosomatidae</td>
<td>610–1170 × 200–500</td>
<td>200–875 × 5–9</td>
<td>3.5–14.5 × 1.5–2.5</td>
</tr>
<tr>
<td><em>O. nutans</em> (Type II) - Japan Urostylidae</td>
<td>720–1170 × 220–360</td>
<td>275–740 × 5–9</td>
<td>7–11 × 1–2</td>
</tr>
<tr>
<td><em>O. nutans</em> (Type I) - Japan Coreidae</td>
<td>950–970 × 250–260</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><em>O. neonutans</em> - Brazil Pentatomidae</td>
<td>630–1200 × 130–360</td>
<td>220–900 × 3–8</td>
<td>6–15 × 1.2–3</td>
</tr>
</tbody>
</table>

Specimens of *O. neonutans* were found parasitizing true bugs belonging to subfamilies Discocephalinae, Edessinae and Pentatominae of Pentatomidae. Although the hosts could not be identified at species rank, they were visibly different and larger than the Japanese hosts of *O. nutans* complex (Fig. 4). Moreover, the host subfamilies are known to be endemic to Neotropical regions, showing the host specificity of *O. neonutans*.

Recent studies have shown a large number of cryptic species in hypocrealean entomogenous fungi (Evans 1982, Evans & Samson 1984, Kaitsu et al. 2013, Sanjuan et al. 2014). Evans et al. (2011, 2018), while studying zombie ant fungi in Brazil, recognized *Ophiocordyceps unilateralis* (Tul. & C. Tul.) Petch (1931: 74) as a species complex. Based on micromorphological differences and host specificity, Evans et al. (2011) described four new *Ophiocordyceps* species growing on different host species of carpenter ants, three of them coexisting in the same area of Atlantic Forest of the State of Minas Gerais. Kobmoo et al. (2012, 2015) and Araújo et al. (2015, 2018) have also demonstrated more than 20 species within *O. unilateralis* complex from Brazil, Colombia, USA, Australia, Japan and Thailand based on both phylogenetic analysis and micromorphological characteristics, associated with host specificity. As proposed by Evans et al. (2011) and Araújo et al. (2015, 2018) within *Ophiocordyceps unilateralis* complex, cryptic species are likely to occur in other species as in *O. nutans*.

Patouillard (1887) mentioned the host of *O. nutans* as a hemipteran adult in the protologue. As early as 1929, Esaki summarized 15 species of true bugs as hosts of Japanese *O. nutans*. The number of host species has reached more than 30 worldwide, belonging to nine families of true bugs (summarized in Shrestha et al. 2017b). The host families show continental distribution. For example, Pentatomidae and Plataspidae are reported as hosts from Asia and Africa but not from the Oceania (New Guinea). Four host families (Acanthosomatidae, Urostylidae, Dinidoridae and Coreidae) are recorded only from Asia, Tesseratomidae only from New Guinea and Pyrrhocoridae and Reduviidae only from Africa (Shrestha et al. 2017b). Although *O. neonutans* shares the same host family Pentatomidae with *O. nutans*, they differ at the subfamily rank.

Besides *O. nutans* and *O. neonutans*, two more species are recorded on adult bugs: *O. pentatomae* (Koval) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007: 45) on *Pentatoma semiannullata* Motschulsky (1860: 501) (Pentatomidae), and *O. sichuanensis* (Z.Q. Liang & B. Wang) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (2007: 46) on an adult pentatomid. *Ophiocordyceps pentatomae* can be easily distinguished from *O. nutans* complex by the lateral position of the fertile part and yellow to brownish stipe, whereas *O. sichuanensis*, only known from holotype, is characterized by a pink stipe and a wide, ellipsoidal head.

**OPHIOCORDYCEPS NEONUTANS SP. NOV.**
Description of *O. nutans* has been exclusively morphology-based. Nevertheless, the phylogenetic work of Sasaki *et al.* (2012) has shown two distinct lineages of this taxon, designated as Type I and Type II. Type I consists of Chinese, Thai and Japanese specimens, which parasitize members of Coreidae. The second lineage (Type II) consists of only Japanese specimens that parasitize members of Pentatomidae, Acanthosomatidae and Urostylididae. The hosts of Type II are closely related to the new Neotropical lineage. Our study supports Sasaki *et al.* (2012), and, in addition, our phylogenetic tree shows that *O. nutans* is paraphyletic, with Brazilian specimens defined as a residual species within the “sphecocephala” clade. *Ophiocordyceps nutans* was originally described from Japan and the selection of a neotype for this species may be done in the near future. We describe Brazilian specimens as a new species based on our phylogenetic study, supported by its opposite geographical location from Japan and the hosts that are endemic to the Neotropical regions. We also urge to restrict *O. nutans* to Japan and nearby regions, and also recommend that the Asian specimens be circumscribed as, at least, two phylogenetically distinct species associated with host families of Hemiptera (Sasaki *et al.* 2012). We also speculate that the so-called morphological *O. nutans* from South America or Africa is the same as *O. nutans* from Japan. We firmly believe in cryptic speciation within *O. nutans* complex.

According to Sasaki *et al.* (2012), genetic variation correlated to host species is possible in some entomogenous fungi. In our case, considering the morphological, ecological, and phylogenetic divergences, we recognize the occurrence of a new species within *O. nutans* complex in Neotropical region, despite the lack of diagnostic morphological characters. Moreover, the phylogeny of African *O. nutans* within the “sphecocephala” clade in association with their host specificity must be addressed in the near future. Recently, Pažoutová *et al.* (2015) have utilized DNA analysis to circumscribe new species within *Claviceps purpurea* (Fr.) Tul. (1853: 45) complex where the morphological characters and host range overlap but differ in geographic distribution.

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**References**


https://doi.org/10.1093/nar/gkh340


https://doi.org/10.1111/j.1365-2311.1982.tb00643.x


https://doi.org/10.1016/S0007-1536(82)80337-5


https://doi.org/10.1371/journal.pone.0017024


https://doi.org/10.1016/j.fuse.2018.01.002


https://doi.org/10.1111/j.1755-0998.2008.02439.x


https://doi.org/10.1016/S0953-7562(09)80536-4


https://doi.org/10.5943/mycosphere/7/11/4


https://doi.org/10.1093/bioinformatics/bts199


https://doi.org/10.1007/s11557-013-0911-9


https://doi.org/10.1093/molbev/mst010


https://doi.org/10.1111/j.1365-294X.2012.05574.x


https://doi.org/10.1016/j.funbio.2014.10.008


https://doi.org/10.1007/s13225-017-0386-0