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The Prevalence of *Scopulariopsis* sp. and *Penicillium* sp. in African Pygmy Hedgehogs (*Atelerix albiventris*) Skins in a Farm in Bogor District of Indonesia

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Abstract

This research aims to detect the prevalence of *Scopulariopsis* sp. and *Penicillium* sp. in African pygmy hedgehogs (*Atelerix albiventris*) bred at Golden Dhonje Farm, Bogor District. Twenty hedgehogs were sampled to detect the presence of *Scopulariopsis* sp. and *Penicillium* sp. The clinical signs in the sampled African pygmy hedgehogs include alopecia, crusty skin, and dermatitis. The presence of the fungus was confirmed by taking skin samples and then culturing them on Potato Dextrose Agar (PDA) supplemented with chloramphenicol and cycloheximide. Macroscopic and microscopic morphology were observed to identify the two fungal genera. Twenty hedgehogs were sampled, and the results showed that eight of them (40%) were infected with *Scopulariopsis* sp., six (30%) were infected with *Penicillium* sp., and four (20%) were co-infected with both species. The total prevalence of hedgehogs affected by these fungi is 90%. This study found differences in infection rates of *Scopulariopsis* sp. and *Penicillium* sp. among different age groups and sexes. This study is the first to isolate *Scopulariopsis* sp. and *Penicillium* sp. from the skins of African pygmy hedgehogs in Indonesia, which are potential zoonotic pathogens. Further research is needed to expand the sample area, identify other types of fungi, and enhance the understanding of fungal diseases in African pygmy hedgehogs, thereby ensuring the safety of these animals in Indonesia.

Keywords

Dermatomycosis, Four-toed hedgehogs, Fungi, Skin

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Introduction

The African pygmy hedgehog is a pet hedgehog species that can carry fungal infections and harm animals and humans. Fungal infections are a major cause of illness and death worldwide, affecting over 150 million people annually and resulting in 1.7 million fatalities (Kainz *et al.*, 2020). The most common fungal infections in humans are dermatomycoses, which are also frequently found in livestock, pets, and wild animals. The causative agents include dermatophytes, *Candida* sp., *Malassezia* sp., and non-dermatophyte molds.

Non-dermatophyte fungi, including *Scopulariopsis* sp. and *Penicillium* sp., can infect humans and animals, causing problems. *Scopulariopsis* sp. and *Penicillium* sp. are two fungi commonly found in the environment, such as soil and plants, and are often considered contaminants (Rodríguez-Andrade *et al.*, 2021; Torres-Garcia *et al.*, 2022; Westblade *et al.*, 2023). *Scopulariopsis* sp. is mainly associated with nail infections (Macura and Skóra, 2015) but can also cause invasive infections in humans (Iwen *et al.*, 2012). Cases of infection in animals have also been reported. *Scopulariopsis* sp. has been reported as the cause of diseases in various animals, such as goats (Ozturk *et al.*, 2009; Ilhan *et al.*, 2016), black calves (Ogawa *et al.*, 2008), dogs (Sri-Jayantha *et al.*, 2019), rats (Yapicier *et al.*, 2020), canaries (Tunç *et al.*, 2022), cats (da Costa *et al.*, 2019), and horses (Apprich *et al.*, 2010; Hampson *et al.*, 2019). On the other hand, *Penicillium* sp. can cause allergies and infections in the cornea, skin, external ear, respiratory system, and urinary tract, and has been reported to cause dermatitis in a cat (Prayuda *et al.*, 2023; Westblade *et al.*, 2023).

The risk of spreading zoonotic diseases from African pygmy hedgehogs is a serious problem. Currently, there is no available information about these two fungal genera that can potentially cause zoonotic diseases from African pygmy hedgehogs in Indonesia. Therefore, research must be conducted to determine the presence of *Scopulariopsis* sp. and *Penicillium* sp. in African pygmy hedgehogs (*Atelerix albiventris*) and their prevalence. This study is expected to be helpful for researchers, providing valuable additional and supporting information regarding fungal infections in the skin of African pygmy hedgehogs, and serving as a basis for future studies. It is also hoped that it will be helpful for hedgehog owners as a reference for animal care management and increase their awareness of fungal zoonotic diseases that can be transmitted from African pygmy hedgehogs.

Materials and Methods

Sample Size and Code

The sample size used in this research consisted of 20 samples, which represent the entire population on the farm. Each sample obtained from pygmy hedgehogs was labeled with a unique code (APH 1–APH 20). This study used the McKenzie technique to take the hedgehog skin sample.

Clinical Symptom Record and Sample Collection

The researchers recorded the current status of the hedgehogs, including their identification, body weight, and length of stay. In addition, the lesions observed on the sampled animals were scored according to Bexton and Nelson (2016). The sample collection was carried out using the McKenzie toothbrush method. The researchers used a sterilized toothbrush to brush the skin between spines or quills, hairs, and the suspected area, including the skin and hair edges of alopecic or crusty areas. The McKenzie brushing sampling using a toothbrush was carried out for at least 3 minutes (Gautam and Bhatia, 2020). Samples were placed into a sterile container, delivered to the laboratory, and can be stored at room temperature (25°C).

Fungal Culture

Fungal culture samples were obtained using the McKenzie toothbrush technique. The toothbrush was gently touched to Potato Dextrose Agar (PDA) supplemented with chloramphenicol and cycloheximide in a circular pattern, starting from the middle area and moving outward. Then, the PDA was incubated at room temperature (22–30°C) for 14 to 28 days. Once the fungal colony has grown, subculturing can be done to obtain a pure culture if more than one type of fungus is observed macroscopically. Subculturing was performed by transferring a small amount of the colony onto a fresh PDA supplemented with chloramphenicol. After obtaining pure cultures, further identification techniques, such as macroscopic and microscopic examinations, can be carried out to determine the species of the cultured fungi.

Fungal Examination

The growth colonies were then examined macroscopically, and the appearance of the isolates, including color, texture, and shape, was noted for identification purposes, particularly characteristics that distinguish them from *Scopulariopsis* sp. and *Penicillium* sp. *Scopulariopsis* sp. isolates can be recognized by their typical appearance, which is white with a glabrous or powdery texture, and light brown with a light tan periphery on the surface, and a tan color on the reverse side. In contrast, *Penicillium* sp. can be identified by its powdery texture and bluish-green surface color, with or without a white margin, and its reverse side can vary in color, ranging from white to yellow, orange, or red (Westblade *et al*., 2023). Microscopic observation of the isolates was carried out using the tape or flag method with Lactophenol Cotton Blue (LPCB) staining. Each isolate was then observed under the microscope at certain magnifications. Fungal identification was done by comparing both genera's macro-morphology and micro-morphology results with those of several fungal identification textbooks and journals. The resulting data from the procedure is processed and analyzed descriptively. The prevalence of each fungus was calculated after its type was identified using the prevalence formula.

Results and Discussion

Twenty hedgehogs (the total population of the farm) were used in this study, and they were in various physical conditions. Most hedgehogs were obtained from outside the farm, but there were also four baby hedgehogs (APH6, APH7, APH8, APH9) born from breeding hedgehogs on the farm. The hedgehogs sampled consist of 9 males and 11 females. Twelve are adult hedgehogs (lived > 2 months, BW > 200 g), while the remaining are four babies (1 week, BW < 20 g) and four juveniles (1 month, BW < 110 g). The average weight of adult hedgehogs is 276 grams, while that of young and juvenile hedgehogs is 55 grams.

Twelve hedgehogs (60%) experienced alopecia, two (10%) of them with a severity level of 2, and ten (50%) of them with a severity level of 1 or the lowest (Figure 1.A). All hedgehogs (100%) had crusty, flaky skin. There were two hedgehogs (10%) who experienced crustiness with a severity level of 3, nine hedgehogs (45%) with a severity level of 2, and nine hedgehogs (45%) with the lowest severity level (Figure 1.B and Figure 1.C). Dermatitis was only experienced by five hedgehogs (25%), consisting of three hedgehogs (15%) with severity level 1 and two hedgehogs (10%) with severity level 2 as open wounds (Figure 1.D). Apart from that, four hedgehogs (20%) were also found to have open wounds that had hardened or dried and swelling on the feet, the area around the face, and the tips of the earlobes.

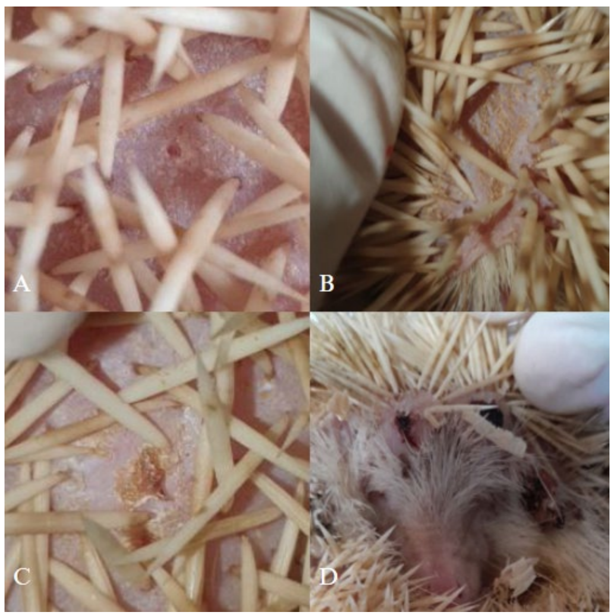

Figure 1. Conditions of level 2 alopecia in APH13 (A), level 2 crusty in APH12 (B) and APH13 (C), and level 2 dermatitis in APH2 (D) of hedgehogs sampled at the farm

Scopulariopsis sp. and *Penicillium* sp. are keratinophilic fungi, which means they can invade and degrade keratinized tissues (Hamm *et al*., 2020; De Macedo and Freitas, 2021; Hamada *et al*., 2024). The tissues in African pygmy hedgehogs that contain keratins include skin, hair, and quills or spines (Crofts and Stankowich, 2021). However, this study reported that *Scopulariopsis* sp. and *Penicillium* sp. isolated from hedgehog skins can also grow in most agar plates. According to Watanabe *et al*. (2022), cutaneous fungal infections are considered to have occurred. They are suspected when five or more of the same colonies grow on a single agar plate medium. Although it should be confirmed with Koch's postulate, there is a possibility that *Scopulariopsis* sp. and *Penicillium* sp. are the causative agents of alopecia, crusty dermatitis, or dermatitis in this study, given the number of colonies and their characteristics that can grow in keratinized tissue.

Forty-one fungal isolates were subcultured due to their morphology, representing *Scopulariopsis* sp. and *Penicillium* sp. There are 16 isolates identified as *Penicillium* sp., and 25 isolates identified as *Scopulariopsis* sp. (Table 1). The media used in this study are PDA with supplementation of chloramphenicol and cycloheximide. Chloramphenicol is used to inhibit the growth of bacteria, whereas cycloheximide is used to inhibit the growth of contaminant saprophytic fungi. This study validated PDA with cycloheximide and chloramphenicol supplementation as media for the isolation of *Scopulariopsis* sp. and *Penicillium* sp. from hedgehog skin samples. These results are similar to those of El-Said *et al*. (2009), who reported the growth of cycloheximide-resistant fungi, including *Scopulariopsis* sp. and *Penicillium* sp..

The macroscopic morphological characteristics of *Scopulariopsis* sp. isolates observed in this study are represented by three isolate codes, namely 13BCC13, 15BCC5, and 18BCC3 (Figure 2 and Table 1). The texture is glabrous to loosely floccose, and the topography is flat, rugose, or with a folded center. The reverse color of the fungal isolates varies, specifically white, tan, and tan with a browner center, which is similarly described by other studies (Westblade *et al*., 2023). The diameter growth of *Scopulariopsis* sp. in the first week ranged from 10–17 mm with an average diameter of 13.56 mm. The rate of growth of *Scopulariopsis* sp. is rapid, as it becomes a mature colony within five days (Westblade *et al*., 2023).

Table 1. Macroscopic morphology of *Scopulariopsis* sp. and *Penicillium* sp. on Potato Dextrose Agar (Sub-culture Plates)

Code of Isolate	Genus/Species	Colony				
		Color	Texture	Reverse Color	Topography	Diameter (mm)
1bcc1	*Penicillium* sp.	G	P	Y	Rf	16
1bcc8	*Penicillium* sp.	G	P	Y	Fl	17
3bcc4	*Penicillium* sp.	Bg	V	W	Fl	24
5bcc3	*Scopulariopsis* sp.	Wt	Gl	T	Rg	12
5bcc5	*Scopulariopsis* sp.	Wt	Lf	W	Rg	12
6bcc4	*Scopulariopsis* sp.	B	Gl	T	Fl	17
7bcc1	*Scopulariopsis* sp.	B	Gl	T	Fl	16
7bcc7	*Scopulariopsis* sp.	Wt	Gl	Yt	Rg	13
7bcc14	*Scopulariopsis* sp.	C	Gl	Wt	Rg	15
7bcc18	*Scopulariopsis* sp.	Wt	Gl	T	Fl	15
7bcc19	*Scopulariopsis* sp.	B	Gl	T	Fl	15
7bcc29	*Scopulariopsis* sp.	Wt	Gl	W	Rg	13
7bcc31	*Scopulariopsis* sp.	B	Gl	T	Fl	24
8bcc7	*Scopulariopsis* sp.	Wt	Gl	T	Rg	14
8bcc14	*Scopulariopsis* sp.	Wt	Gl	W	Rg	13
8bcc16	*Scopulariopsis* sp.	B	Lf	T	Fl	15
8bcc26	*Scopulariopsis* sp.	B	Lf	T	Fl	15
9bcc16	*Scopulariopsis* sp.	B	Lf	T	Fl	14

Code of Isolate	Genus/Species	Colony				
		Color	Texture	Reverse Color	Topography	Diameter (mm)
10bcc1	*Penicillium* sp.	Bg	P	W	Fl	22
10bcc2	*Penicillium* sp.	Bg	P	W	Fl	20
10bcc3	*Penicillium* sp.	G	P	W	Fl	15
10bcc12	*Scopulariopsis* sp.	B	Lf	Tb	Fl	13
11bcc1	*Penicillium* sp.	Bg	V	W	Fl	20
11bcc3	*Scopulariopsis* sp.	B	Lf	Wt	Rg	15
12bcc8	*Penicillium* sp.	G	P,	W	Fl	13
13bcc1	*Penicillium* sp.	G	P	W	Rf	17
13bcc4	*Penicillium* sp.	G	P, F	W	Fl	19
13bcc6	*Scopulariopsis* sp.	Wt	Gl	W	Fd	13
13bcc13	*Scopulariopsis* sp.	B	Lf	T	Fd	11
14bcc1	*Scopulariopsis* sp.	B	Lf	T	Fd	10
15bcc1	*Penicillium* sp.	G	P	Y	Fl	14
15bcc3	*Penicillium* sp.	Bg	Lf	W	Fl	20
15bcc5	*Scopulariopsis* sp.	W	Lf	W	Fd	12
16bcc1	*Penicillium* sp.	Bg	P	Y	Rg	15
17bcc1	*Scopulariopsis* sp.	B	Lf	T	Fl	13
17bcc2	*Scopulariopsis* sp.	B	Lf	T	Fl	11
18bcc2	*Scopulariopsis* sp.	W	Lf	W	Fd	11
18bcc3	*Scopulariopsis* sp.	B	Lf	T	Fd	16
19bcc1	*Penicillium* sp.	Bg	Lf	W	Fl	20
20bcc1	*Penicillium* sp.	Bg	P, Lf	W	Fl	19
20bcc2	*Penicillium* sp.	G	P	R	Fl	16

Note: **Color:** B: Brown; Bg: Bluish green; C: Cream; G: Green; R: Red; T: Tan; Tb: Tan-brown; W: White; Wt: White-tan; Y: Yellow; Yt; Yellow-tan; **Texture:** P: Powdery; V: Velvety; F: Floccose; Lf: Loosely floccose; **Topography:** Rf: Radial fold; Fl: Flat; Rg: Rugose; Gl: glabrous; Fd: folded in the middle. **Diameter**: Diameter of 7-day-old fungal colonies.

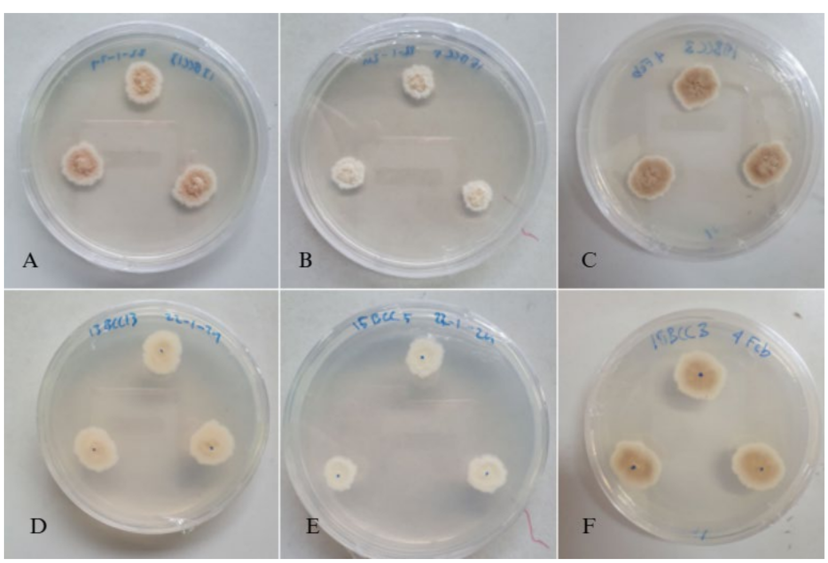

Figure 2. Macroscopic morphology of *Scopulariopsis* sp. colonies on day 7 on Potato Dextrose Agar: the surface of 13BCC13 (A), 15BCC5 (B), and 18BCC3 (C) isolates; the reverse of 13BCC13 (D), 15BCC5 (E), and 18BCC3 (F) isolates.

Further microscopic observation was conducted using a Lactophenol Cotton Blue (LPCB) stain. *Scopulariopsis* sp. exhibits a microscopic structure similar to that of *Penicillium* sp. The structure consists of septate hyphae, branched annelids, and smooth- and rough-walled conidia in chains consistently found in most isolates (Figure 3). The annelids may be solitary or in a group that forms a finger-like conidiophore structure.

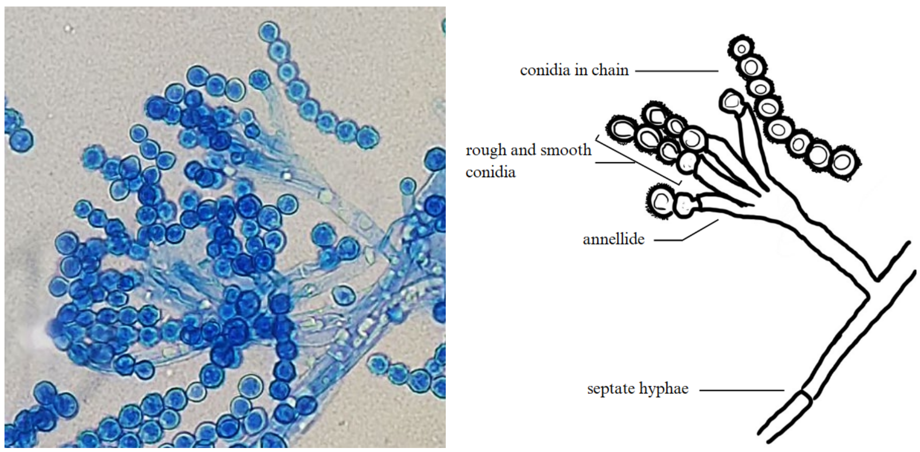

Figure 3. Microscopic Observation of *Scopulariopsis* sp. (7BCC18) under a microscope at 400x magnification using LPCB stain (left) and a schematic representation of its morphology (right), illustrated by the author (Satria Tegar Rahmadani)

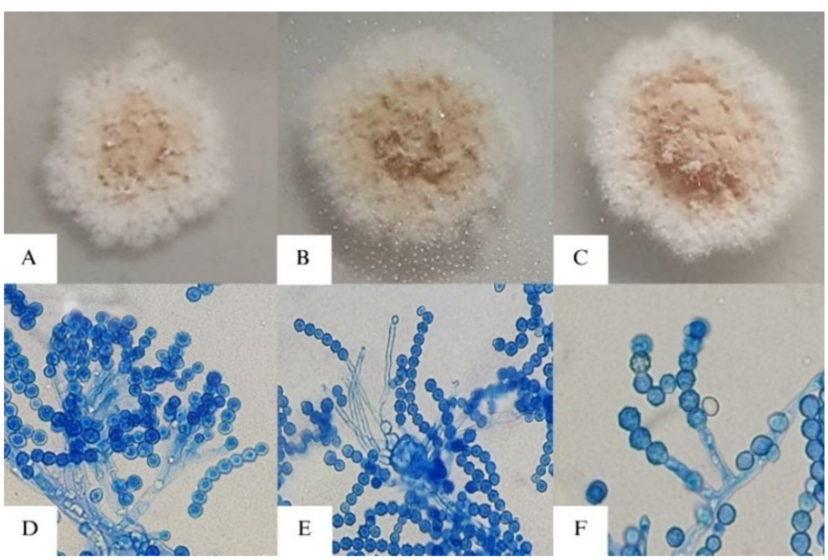

Figure 4. Macroscopic (A, B, C) and microscopic (D, E, F) morphology of *Scopulariopsis brevicaulis* in 7BCC18 (A, D), 8BCC26 (B, E), and 10BCC12 (C, F) on day 7 at 400x magnification. The fungi were grown on Potato Dextrose Agar.

Based on macro- and micro-morphological observations, all the *Scopulariopsis* species observed in this study were identified as *Scopulariopsis brevicaulis*. Apart from that, many spores or conidia are formed in chains with a smooth and rough texture and relatively visible size, which is one characteristic of *Scopulariopsis* sp. (Figure 4).

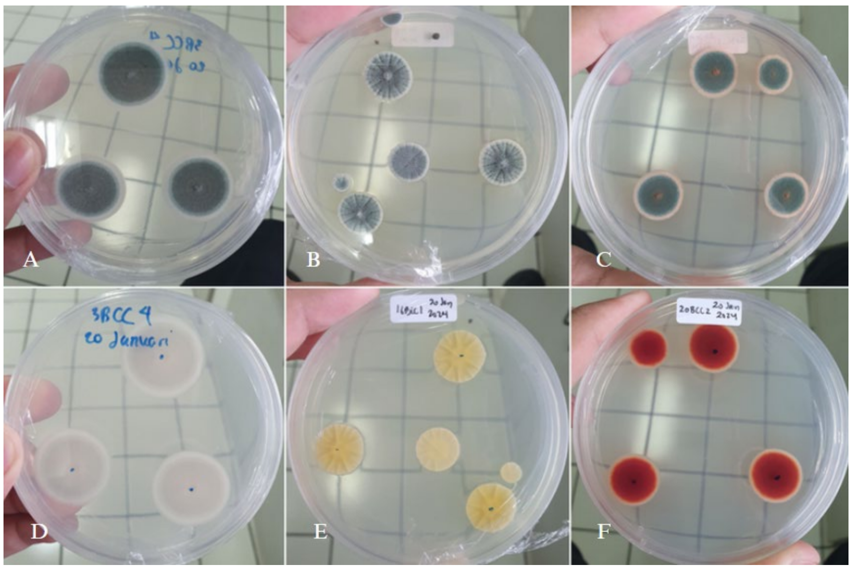

Figure 5. Macroscopic morphology of *Penicillium* sp. colonies on day 7 on Potato Dextrose Agar; reverse and surface of 3BCC4 (A, D), 16BCC1 (B, E), and 20BCC2 (C, F)

The macroscopic morphological characteristics of *Penicillium* sp. isolates observed in this study (Table 1) are represented by three isolate codes, namely 3BCC4, 16BCC1, and 20BCC2. Figure 5 shows that *Penicillium* sp. isolates have a green or bluish-green color. The texture is powdery or floccose with flat or rugose topography. The color of the reverse fungi is varied, ranging from white to yellow or even red. Isolate 3BCC4 has wider sporulation than the other two and a bluish-green surface, a powdery texture with a light margin, and a white reverse. Isolate 16BCC1 exhibits a bluish-green surface color, a velvety texture, rugose topography, and a yellowish reverse color.

Microscopically, *Penicillium* sp. isolates showed a typical structure sequentially: septate hyphae, stipe, branches like metulae, branched phialide, and conidia formed in a chain at the end of the phialide (Figure 6). Several branching types were found, such as divaricate, biverticillate (Figure 6), and terverticillate. The type of conidiophore branching pattern varied among species (Samanta, 2015). Almost all predominant conidiophores of the *Penicillium* sp. isolates observed exhibit a biverticillate branching pattern, except for isolate 19BCC1, which has a divaricate conidiophore pattern.

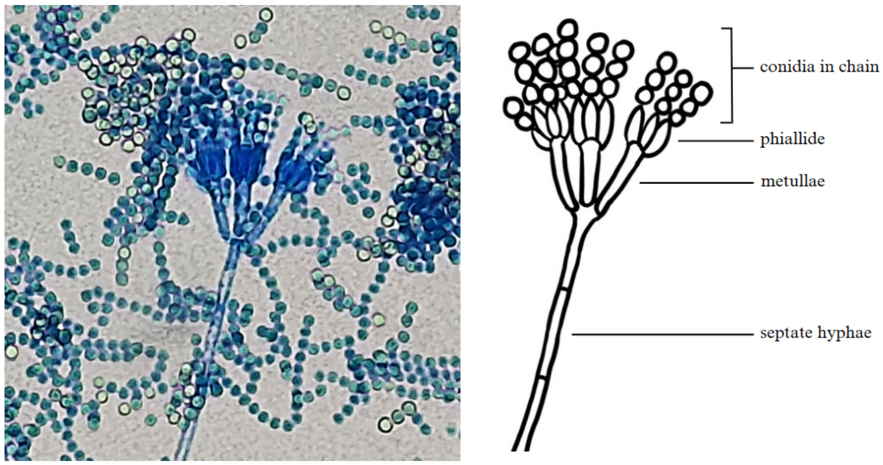

Figure 6. Microscopic (left) and schematic (right) morphology of biverticillate *Penicillium* sp. in isolate 15BCC1 under the microscope at 400x magnification. The schematic figure was illustrated by the author (Satria Tegar Rahmadani).

Isolate 20BCC2 shows a significant reverse color difference. It is the only isolate that shows red pigmentation on its reverse. Its surface shows a green, powdery texture with flat topography. A green, flat topography, powdery texture with red reverse pigmentation, and biverticillate conidiophore structure are the characteristics of *Penicillium marneffei* and *P. purpurogenum* (Vi and Kh, 2017; Westblade *et al.*, 2023). Isolate 20BCC2 shows these characteristics in its macroscopic and microscopic morphology (Table 1). *Penicillium marneffei* is a dimorphic fungus that can convert into the yeast phase at 37°C (Westblade *et al.*, 2023). However, to confirm 20BCC2 as a dimorphic fungus, the procedure of subculture and incubation at 37°C for 7 to 14 days, followed by further microscopic observation, should be carried out to confirm yeast colony formation. Previous research has shown that cycloheximide in agar media can inhibit the growth of *Penicillium marneffei* and *P. purpurogenum* (Atalay *et al.*, 2016; Westblade *et al.*, 2023).

This study reported the single and co-infection of *Scopulariopsis* sp. and *Penicillium* sp. in African pygmy hedgehogs. Among the 20 sampled African pygmy hedgehogs, the single-infection prevalence of *Scopulariopsis* sp. and *Penicillium* sp. is 40% (8/20) and 30% (6/20), respectively. *Scopulariopsis* sp. and *Penicillium* sp. co-infections were observed in 20% (4/20) of hedgehogs, namely APH10, APH11, APH13, and APH15. The total prevalence of *Scopulariopsis* sp. and *Penicillium* sp. infection in this study is 90% (18/20). As per the number of isolates on BCC media, two hedgehogs in samples APH2 and APH4 were uninfected by both genera. In this study, male African pygmy hedgehogs have a prevalence of *Scopulariopsis* sp. and *Penicillium* sp. infections of 55% (5/9). Meanwhile, the prevalence of *Scopulariopsis* sp. and *Penicillium* sp. in female African pygmy hedgehogs was 63% (7/11) and 45% (5/11), respectively. Most young hedgehogs were infected by *Scopulariopsis* sp. (87.5%), while only some adults were infected (41.6%). *Penicillium* sp. infection was high in adult hedgehogs (75%) but low in young hedgehogs (25%).

This report supported a previous study by Aftab *et al*. (2023) that successfully isolated *Scopulariopsis* sp. from 12.5% (6/48) of other hedgehog breeds. *Scopulariopsis* sp. infections have also been reported with a prevalence of 5% (10/200) in canaries, 50% (16/32) in horses, and 10% in humans (Rosa *et al*., 2003; Fitzpatrick *et al*., 2018; Tunç *et al*., 2022). *Scopulariopsis* sp. is considered pathogenic in animals as it is the causative agent of hyperkeratosis in black calves. *Scopulariopsis* sp. causes hair loss and dermal lesions in goats (Ozturk *et al*., 2009; Ilhan *et al*., 2016) and rats (Yapicier *et al*., 2020). It was identified as the causative agent of rhinosinusitis in canine patients (Sri-Jayantha *et al*., 2019) and sino-orbital infection in feline patients (da Costa *et al*., 2019). *Scopulariopsis* sp. also caused mortality with invasive infections in canaries (Tunç *et al*., 2022).

In addition, *Penicillium* sp. has also been reported as the second-highest skin microflora of wild European hedgehogs, with a prevalence of 74.7% (76/102) (Molina-López *et al*., 2012), 13% (2/15) in dogs (Sudipa and Gelgel, 2022), and 9.45% (12/127) in cats (Sattasathuchana *et al*., 2020). The prevalence of *Scopulariopsis* sp. and *Penicillium* sp. varies with each host and environmental condition. The differences in prevalence in each animal species are influenced by their daily behavior. African pygmy hedgehogs are nocturnal animals, and they prefer to burrow underground during the day to create a dark environment. They also have the habit of digging to find food (Wissink-Argilaga, 2020). If fungal spores are on the ground or bedding, they will cover most of the hedgehog's body. Therefore, hedgehogs have a higher risk of contracting saprophytic fungi, including *Scopulariopsis* sp. and *Penicillium* sp., than other animals because of their habit of burying themselves.

For this study, hedgehogs were housed on a farm in individual, mating, or nursery cages with wood shavings and/or rice husks as their bedding. Therefore, several possibilities of the origin of these two fungal genera (*Scopulariopsis* sp. and *Penicillium* sp.) are from bedding like wood shavings (Gomes *et al*., 2022), dung (Woudenberg *et al*., 2017), feeds (Macura and Skóra, 2015; Witaszak *et al*., 2020), drinking water (Babič *et al*., 2017), caretakers, other hedgehogs (Pignon and Mayer, 2011), or from the surrounding environment, including air (Viegas *et al*., 2013; Macura and Skóra, 2015), soil, and animal enclosures (Visagie *et al*., 2014; Eze *et al*., 2019; Yapicier *et al*., 2020). According to the farm keeper, the African pygmy hedgehogs on the farm have never been bathed. Sanitation is only performed on their cage, including changing bedding every 1 to 2 weeks and providing water *ad libitum*. On the farm, dry food (commercial cat food) is offered regularly, twice daily. If the wood shavings used for bedding, feed, or a source of drinking water have been contaminated by fungi, they will most likely contaminate the animals. If the hedgehogs were taken from the wild, it could be that the animals have been contaminated since they were first kept. After the hedgehogs are infected, they can spread the infection to other hedgehogs directly or through the air, as the fungus is airborne. On the farm, the hedgehog cages are located in one indoor area alongside cages for various species, including sugar gliders, chickens, rabbits, and geckos. Even though each animal cage was still separated from the others, there is still a possibility that the spores of these fungi can communicate with this animal through the air. Therefore, the sanitation of the animals and their cage is necessary to prevent and overcome the fungal infection problem in the animals.

Conclusion

The African pygmy hedgehog is a pet breed that can carry fungal infections, which can harm both animals and humans, including *Scopulariopsis* sp. and *Penicillium* sp. This study detected *Scopulariopsis* sp. and *Penicillium* sp. on the skin of African pygmy hedgehogs, which were identified based on their macroscopic and microscopic morphology, and presented their prevalence. The prevalence of single *Scopulariopsis* sp. and *Penicillium* sp. infections in this study, respectively, is 40% (8/20) and 30% (6/20), with 20% (4/20) of the sampled animals being coinfected with both fungal genera.

Approval of Ethical Commission

Ethical clearance was waived because the study did not include any experimental procedures that might cause pain, suffering, distress, or long-term harm equal to or greater than that caused by needle use. The sample collection was performed using a non-invasive technique, the McKenzie toothbrush method.

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Author's Contribution

Conceptualization: STR, NGB, N; Methodology: STR, NGB; Validation: NGB, N; Formal analysis: NGB, N; Investigation: STR, NGB; Resources: NGB; Data Curation: NGB, N; Writing - Original Draft: STR; Writing - Review & Editing: NGB, N; Visualization: STR; Supervision: NGB, N.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data is already presented in the paper and can be accessed by the readers.

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