Taxonomic reexamination of *Auricularia* specimens based on updated morphological criteria

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Abstract

Auricularia specimens deposited in the National Museum of Nature and Science, Japan, were observed to reexamine their species identification based on updated taxonomic criteria, and to verify the taxonomy and distribution of Auricularia spp. in Japan. Four of the ten specimens previously identified as A. auriculajudae were reidentified as A. americana (1), A. cornea (1), and A. villosula (2) based on morphological criteria such as medullae, abhymenial hairs, and basidiospores. The remaining six specimens could not be identified at the species level due to the lack of distinguishing morphological characteristics. Two of the five specimens previously considered A. minor were identified as different species due to basidiospore size discrepancy, but the remaining two specimens and the type specimen were in poor condition, and their basidiospores could not be properly observed. Three of the nine specimens previously identified as A. polytricha were reidentified as A. cornea based on morphological criteria. The remaining six specimens were not identified as A. polytricha. The type specimens of A. polytricha f. leucochroma and A. polytricha f. tenuis were in poor condition, and their basidiospores could not be properly observed. Reexaminations of existing specimens based on recent taxonomic criteria will contribute to updating the taxonomy and distribution of Auricularia spp. in Japan.

Key Words: Auriculariales, herbarium, identification, morphology, taxonomy

Introduction

Species of the genus *Auricularia* (Auriculariales, Agaricomycetes, Basidiomycota) occur as wood-decomposing fungi in forest ecosystems, and the fruiting-bodies of several species are widely used as edible mushrooms in Asia. The classification of *Auricularia* species was formerly based on macroscopic characteristics, such as the color and external shape of basidiocarps¹. Lowy proposed a classification of *Auricularia* species on the internal stratification of hyphae in basidiocarps², and Kobayasi examined Asian *Auricularia* species based on the same criterion³. Weiß and Oberwinkler also reexamined the genera in Auriculariales based on molecular phylogenetic analyses⁴. In recent years, the species taxonomy of *Auricularia* has been revised based on molecular phylogenetic relationships and morphological characteristics⁵⁻⁹.

As the results of revised taxonomic standards based on phylogenetic relationships, several new Auricularia species have been described in Asia, and some specimens have been reidentified as different species. Wu et al. conducted taxonomic reexaminations of the Auricularia auricula-judae complex collected from China,

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and A. heimuer F. Wu, B.K. Cui & Y.C. Dai, A. minutissima Y.C. Dai, F. Wu & Malysheva, and A. tibetica Y.C. Dai & F. Wu have been described as new species ^{6, 7)}. Based on specimens collected from Thailand, Bandara et al. described A. thailandica Bandara & K.D. Hyde and A. asiatica Bandara & K.D. Hyde as new species, and reported A. villosula Malysheva and A. cornea Ehrenb. for the first time from Thailand ^{8, 9)}.

In Japan, taxonomic studies based on morphological characteristics of Auricularia species have been carried out by Yasuda¹⁰⁻¹²⁾ and Kobayasi^{3, 13, 14)}. Yasuda described A. papyracea Yasuda as a new species based on a specimen collected in Japan¹¹⁾. Kobayasi described A. minor Kobayasi as a new species based on a specimen collected from Taiwan, and stated that this species is also distributed in Japan³⁾. Shirouzu et al. reexamined the taxonomy of Japanese Auricularia species based on molecular phylogeny and morphological observations¹⁵⁾. As a result, 26 specimens and cultures, including specimens previously identified as A. auricula-judae (Bull.) Wettst. based on morphological criteria, have been reidentified as A. heimuer, A. minutissima, A. villosula, and A. thailandica. These results support the hypothesis that A. auricula-judae sensu stricto is distributed only in Europe^{6, 16)}. Additionally, another 26 specimens and cultures, including specimens previously identified as A. polytricha (Mont.) Sacc., have been reidentified as A. cornea¹⁵⁾.

As described above, the recent taxonomic reexaminations of *Auricularia* species based on phylogenetic relationships have led to the revision of morphological criteria for species identification. Therefore, taxonomic reexaminations, together with the molecular phylogenetic studies, based on the updated criteria are necessary for the herbarium specimens previously identified as *Auricularia* spp. In this study, we aimed to reexamine the identification of the *Auricularia* specimens deposited in the National Museum of Nature and Science, Japan, by microscopic observation and morphological comparison. We attempted to extract DNA from the pieces of specimens. PCR and sequencing of the ITS (internal transcribed spacer) region of nrDNA (ITS1-5.8S rDNA-ITS2) were attempted using the same method in the previous study by Shirouzu et al.¹⁵, but the target sequences could not be obtained in all specimens probably because of age and poor storage conditions. Therefore, in the present study, we solely focused on a careful examination of morphological characters, following the most current species standards.

Materials and Methods

Twenty-six specimens of *Auricularia* were selected from the collections of the National Museum of Nature and Science, Japan (TNS, Tsukuba, Ibaraki) with recorded specimen information such as collection site and date (Table 1). The dried basidiocarps were rehydrated in Milli-Q water, and morphological observations and measurements were made macroscopically and with a stereomicroscope (Stemi 305, Carl Zeiss, Göttingen, Germany). Abhymenial hairs, medullae, basidia, and basidiospores were observed using a biological microscope (Axiophot, Carl Zeiss, Göttingen, Germany). Abhymenial hairs and medullae were observed with 10× or 20× objective lenses, while basidiospores and basidia were observed with a 40× objective lens. For the observation of basidiospores and basidia, 10–15µm thick sections of basidiocarps were prepared using a freezing microtome (Litratome, REM-710, Yamato Kohki, Asaka, Saitama, Japan). When basidiospores were not observed in the sections, they were collected from the surfaces of the hymenia, and microscopic preparations were made. For the observation of medullae, thicker sections (>100µm) were prepared by manual sectioning. A 5% KOH aqueous solution was used as the mounting medium. The obtained morphological characteristics were compared with the descriptions of previous studies for species identification^{5-9, 15, 16}.

Identification in previous study	TNS-F No.	Host	Locality	Date	Collector	Reference	Identification in this study
Auricularia auricula-judae	427	1	Japan, Chiba	22 Apr. 1964	Y. Kobayasi		A. villosula
A. auricula-judae	6621	I	Japan, Tokyo	Nov. 1940	Y. Kobayasi		not A. auricula-judae s. str.
A. auricula-judae	6635	ı	Japan, Oita	7 Dec. 1919	N. Nakayama	ı	not A. auricula-judae s. str.
A. auricula-judae	6640	ı	Japan, Bonin Islands	1 Jul. 1912	S. Hori	10)	not A. auricula-judae s. str.
A. auricula-judae	195314	Picea jezoensis	Russia, Sakharin	30 Jul. 1940	Y. Kobayasi	13)	A. amreicana
A. auricula-judae	196668	I	Taiwan, Kanko	25 Mar. 1940	Y. Kobayasi	13)	not A. auricula-judae s. str.
A. auricula-judae	196708	Robinia pseudoacacia	Japan, Wakayama	20 Jul. 1918	Ui		A. cornea
A. auricula-judae	196710	ı	Japan, Nagano	23 Sep. 1923	Kohinata		A. auricula-judae complex
A. auricula-judae	196790		Japan, Hyogo	20 Nov. 1916	Matsushima	ı	A. auricula-judae complex
A. auricula-judae	196792		Japan, Fukushima	5 Sep. 1921	Narita	ı	A. villosula
A. minor	428	I	Taiwan	Dec. 1979	Y. Kobayasi		Poor specimen condition
A. minor	433	ı	Papua New Guinea, Rabaul	29 Dec. 1969	Y. Kobayasi	ı	not A. minor
A. minor	434^{a}	Broad-leaved tree	Taiwan	Aug. 1977	Y. Kobayasi	3)	Poor specimen condition
A. minor	51392	ı		4 Oct. 1981	Y. Kobayasi		Poor specimen condition
A. minor	196884	I	Taiwan	I	Y. Kobayasi	ı	not $A.\ minor$
A. polytricha	6555	ı	Japan, Miyazaki	Aug. 1950	Y. Kobayasi		A. cornea
A. polytricha	6569	ı	Japan, Kagoshima	Nov. 1953	Y. Kobayasi		not A. polytricha or A. nigricans
A. polytricha	6574	ı	Taiwan, Kanko	25 Mar. 1940	Y. Kobayasi	13)	not A. polytricha or A. nigricans
A. polytricha	6099	ı	Taiwan, Kanko	25 Mar. 1940	Y. Kobayasi	13)	not A. polytricha or A. nigricans
A. polytricha	6610	I	Japan, Miyazaki	Aug. 1950	Y. Kobayasi	ı	not A. polytricha or A. nigricans
A. polytricha	6642	ı	Japan, Bonin Islands	23 Nov. 1936	Y. Kobayasi	13)	A. cornea
A. polytricha	6650	ı	Japan, Tokyo	ı	Y. Kobayasi		not A. polytricha or A. nigricans
A. polytricha	195396		Japan, Bonin Islands	28 Mar. 1936 Y. Kobayasi	Y. Kobayasi	13)	not A. polytricha or A. nigricans
A. polytricha	196722	ı	Japan, Hachijojima Island	24 Mar. 1936	Y. Kobayasi	13)	A. cornea
A. polytricha f. leucochroma	193009^{a}	Hibiscus glaber	Japan, Bonin Islands	Nov. 1936	M. Okabe	3, 13)	Poor specimen condition
A. polytricha f. tenuis	6563^{a}	ı	Micronesia, Saipan	21 Aug. 1936	Y. Kobayasi	13)	Poor specimen condition

Table 1. Auricularia specimens observed in this study.

Results and Discussion

Identified specimens

Species identification of 26 specimens were reexamined in this study (Table 1). Of these, seven specimens were reidentified as belonging to three different species - one specimen as *A. americana*, two as *A. villosula*, and four as *A. cornea*. The morphological descriptions of these three species are as follows.

Auricularia americana Parmasto & I. Parmasto ex Audet, Boulet & Sirard, in Boulet, Les Champignons des Arbres de l'Est de l'Amérique du Nord: 287, 2003. (Figs. 1, 2)

Basidiocarps cupulate, with undulate margin, hard-gelatinous to cartilaginous, 2.3–2.6 cm broad; upper surface velvety, brown; hymenial surface smooth, dark brown. Medullae pale brown, 95.5–153 µm broad. Internal hyphae branched, septate, thin-walled, hyaline, with clamp connections, 1–2.5 µm diam. Abhymenial hairs gregarious, cylindrical, flexuous, thick-walled with a narrow rumen, apically acute to obtuse, hyaline with pale brown pigmentation at the base, 74.5–144 × 4–6 µm. Basidia clavate to cylindrical, transversely 3-septate, hyaline, 52.5–67 × 5–7 µm. Basidiospores allantoid to reniform, thinwalled, hyaline, 14–17 × 5–6.5 µm (15.7 × 5.8 µm on average, n = 10), 1/w 2.6–3.3 (2.8 on average, n = 10).

Specimen used for description: RUSSIA, Sakhalin, on Picea jezoensis, 30 Jul. 1940, Y. Kobayasi, TNS -F-195314 (as A. auricula-judae).

Auricularia americana is a species reported in North America and Asia⁷). The molecular phylogenetic analysis by Looney et al. suggested that this species can be divided into two lineages, one on broad-leaved trees and the other on coniferous trees⁵). Wu et al. described the lineage without medulla and on broad-

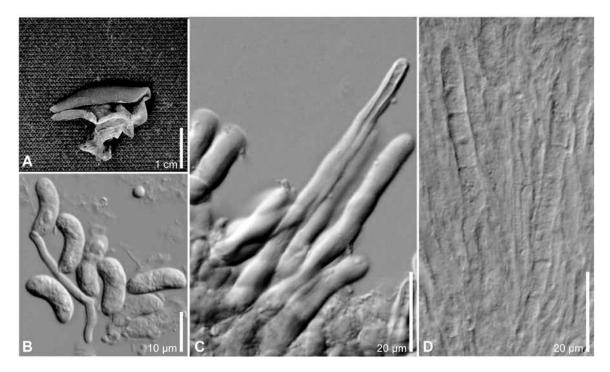


Fig. 1 Auricularia americana TNS-F-195314. A. basidiocarp, B. basidiospores, C. abhymenial hairs, D basidia.

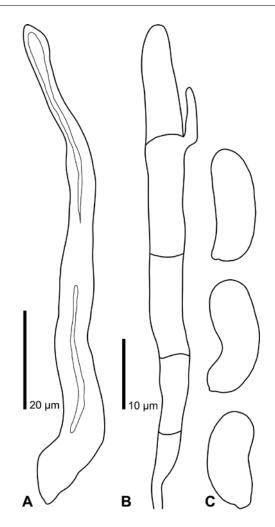


Fig. 2 Auricularia americana TNS-F-195314. A.abhymenial hair, B. basidium, C. basidiospores

leaved trees as A. angiospermarum and considered the lineage on coniferous trees as A. americana s. str.⁷. TNS-F-195314 was identified as A. americana s. str. because its habitat and morphological characteristics were consistent with the description by Wu et al.: abhymenial hairs less than 150 μ m long, distinct medullae, basidia less than 75 μ m long, basidiospores reaching more than 15 μ m in length, and inhabiting a coniferous tree⁷. The species with morphological characteristics similar to A. americana s. str. are A. angiospermarum, A. auricula-judae s. str., A. heimuer, and A. tibetica. Of these species, A. angiospermarum, A. auricula-judae s. str., and A. heimuer differ from A. americana in that they inhabit broad-leaved trees⁷. Auricularia tibetica (70–103 μ m) is distinguished from A. americana (<75 μ m) by the longer basidium length⁷.

Auricularia cornea Ehrenb., in Nees von Esenbeck, Horae Phys. Berol.: 91, 1820. (Figs. 3, 4)

Basidiocarps cupulate, with undulate margin, hard-gelatinous to cartilaginous, 2.6–3.1 cm broad; upper surface velvety, dark brown; hymenial surface smooth, dark brown. Medullae pale brown to brown, 114–189 μ m broad. Internal hyphae branched, septate, thin-walled, hyaline, with clamp connections, 1.5–5 μ m diam. Abhymenial hairs gregarious, cylindrical, flexuous, thick-walled with a narrow rumen, apically acute to obtuse, hyaline with pale brown pigmentation at the base, 155–376 × 5.5–8.5 μ m. Basidia clavate to cylindrical, transversely 3-septate, hyaline, 42.5–53 × 3.5–6.5 μ m. Basidiospores allantoid to reniform, thin-walled, hyaline, $13.5-17.5 \times 5.5-6 \ \mu m \ (15.5 \times 6 \ \mu m \ on \ average, n = 9)$, $1/w \ 2.5-3 \ (2.5 \ on \ average, n = 9)$.

Specimen used for description: JAPAN, Miyazaki Pref., Aug. 1950, Y. Kobayasi, TNS-F-6555 (as A. polytricha).

Other specimens examined: Wakayama Pref., on *Robinia pseudoacacia*, 20 July 1918, Ui, TNS-F-196708 (as *A. auricula-judae*); Tokyo, Hachijojima Isl., 24 March 1936, Y. Kobayasi, TNS-F-196722 (as *A. polytricha*); Tokyo, Bonin Isls., 23 Nov. 1936, Y. Kobayasi, TNS-F-6642 (as *A. polytricha*).

Auricularia cornea are widely distributed in Asia⁵⁾. The above four specimens were identified as A. cornea because their morphological characteristics were consistent with the descriptions by Looney et al. and Shirouzu et al.: abhymenial hairs reaching more than 300 μ m but not 600 μ m in length, distinct medullae, and basidiospores reaching more than 17 μ m in length^{5, 15)}. Morphologically similar species to A. cornea are A. polytricha and A. nigricans (=? A. polytricha)⁵⁾. These two species differ from A. cornea in the length of their abhymenial hairs, which reach more than 600 μ m in A. polytricha and more than 650 μ m in A. nigricans^{3, 5)}.

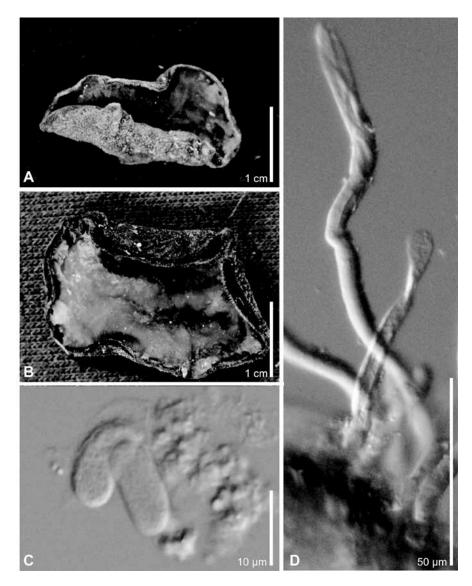


Fig. 3 Auricularia cornea TNS-F-6555. A, B. basidiocarps, C. basidiospores, D. abhymenial hairs.

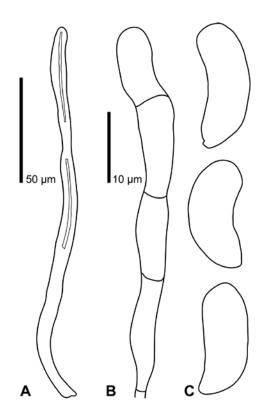


Fig. 4 Auricularia comea TNS-F-6555. A. abhymenial hair, B. basidium, C. basidiospores.

Shirouzu et al. reidentified the Japanese samples previously identified as A. polytricha to A. cornea¹⁵. In the present study, three specimens identified as A. polytricha were also reidentified as A. cornea. One specimen, which has been considered to be A. auricula-judae, was reidentified as A. cornea.

Auricularia villosula Malysheva, in Malysheva & Bulakh, Nov. Sist. Niz. Rast. 48: 174, 2014. (Figs. 5, 6)

Basidiocarps cupulate, with undulate margin, hard-gelatinous to cartilaginous, 1.9–2.7 cm broad; upper surface smooth, light brown; hymenial surface smooth, bright yellow. Medullae absent or indistinct. Internal hyphae branched, septate, thin-walled, hyaline, with clamp connections, 2.5–4.5 μ m diam. Abhymenial hairs gregarious, cylindrical, flexuous, thick-walled with a narrow lumen, apically obtuse, hyaline with pale brown pigmentation at the base, 43.5–86.5 × 5–9.5 μ m. Basidia clavate to cylindrical, transversely 3-septate, hyaline, 44.5–71.5 × 3.5–6.5 μ m. Basidiospores allantoid to reniform, thin-walled, hyaline, 10–15.5 × 5.5–7 μ m (12.8 × 6.1 μ m on average, n = 8), length/width 1.51–2.54 (2.1 on average, n=8).

Specimen used for description: JAPAN, Chiba Pref., 22 Apr. 1964, Y. Kobayasi, TNS-F-427 (as A. auricula-judae).

Other specimens examined: Fukushima Pref., 5 Sept. 1921, Narita, TNS-F-196792 (as A. auricula-judae).

Auricularia villosula is a species described based on a specimen collected from the Russian Far East ¹⁶. This species has also been reported in China, Thailand, and Japan^{6, 9, 15}. TNS-F-427 and 196792 were identified as A. villosula because their morphological characteristics were consistent with the descriptions

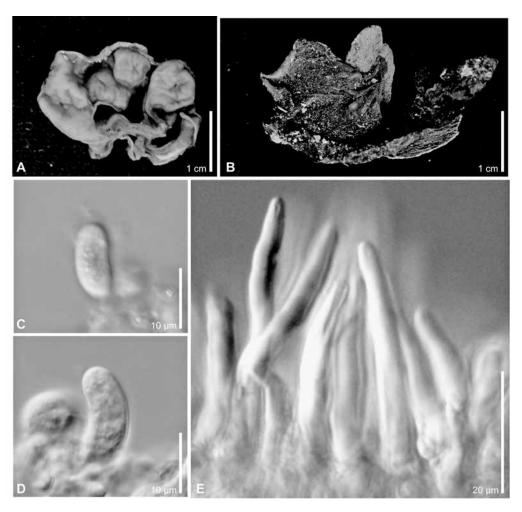


Fig. 5 Auricularia villosula TNS-F-427. A, B. basidiocarps, C, D. basidiospores, E abhymenial hairs.

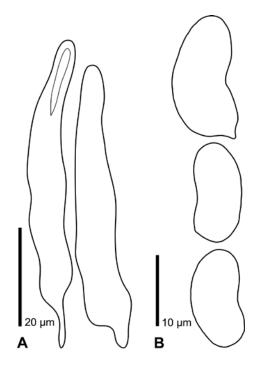


Fig. 6. Auricularia villosula TNS-F-427. A. abhymenial hairs, B. basidiospores.

by Malysheva and Bulakh, Wu et al., and Shirouzu et al.: abhymenial hairs less than 100 μ m long, absent or indistinct medullae, and basidiospores reaching more than 15 μ m in length ^{6, 15, 16)}. Auricularia thailandica is morphologically similar to A. villosula, which has short abhymenial hairs of less than 80 μ m. Auricularia thailandica is distinguished from A. villosula in its distinct medullae, basidia less than 50 μ m long, basidiospores less than 14 μ m long, and has been reported only in Okinawa in Japan^{8, 15)}. Similar species with no or indistinct medullae and similar-sized basidiospores are A. americana s. str., A. angiospermarum, and A. auricula-judae s. str. However, these species are distinguished from A. villosula by their abhymenial hairs reaching more than 100 μ m in length ⁷⁾.

Specimens previously considered as Auricularia auricula-judae

All 10 specimens previously considered as *A. auricula-judae* were demonstrated not to be *A. auricula-judae* s. str. Of these specimens, four were identified as different species, as described above. Three of the remaining six specimens (TNS-F-6621, 6640, 196668) had abhymenial hairs with a maximum length of approximately 100 μ m, which is shorter than the length of *A. auricula-judae* s. str. (60–200 μ m, Malysheva and Bulakh; 100–150 μ m, Wu et al.)^{7, 16)}. TNS-F-6635 exhibited abhymenial hairs of 166–555 μ m in length, longer than those of *A. auricula-judae* s. str. These four specimens were considered different from *A. auricula-judae* s. str. However, due to poor specimen conditions, sufficient numbers of basidia (TNS-F-6621, 6640) and basidiospores (TNS-F-6635, 6640) could not be measured. For TNS-F-196668, no species with corresponding morphological characteristics were found among the previously known species. The remaining two specimens (TNS-F-196710, 196790) have morphological characteristics of several species in the *A. auricula-judae* complex, and could not be identified to the species level.

Specimens previously considered as Auricularia minor

Of the five specimens previously considered as *A. minor*, two were demonstrated not to belong to this species. The basidiospores of TNS-F-433 were longer (9–14.5 µm) than the original description of *A. minor* $(7-8 \mu m)^{3}$. The basidiospores (9–12 µm) and abhymenial hairs (89–137 µm) of TNS-F-196884 were longer than the basidiospores (7–8 µm) and abhymenial hairs (30–80 µm) in the original description ³⁾. These two specimens were considered to be different species from *A. minor*, but no species with corresponding morphological characteristics could be found among previously known species. TNS-F-428 and 51392 were not identified because sufficient numbers of basidiospores could not be measured due to poor specimen conditions. The type specimen of *A. minor*, TNS-F-434, was in poor condition, and sufficient numbers of basidiospores could not be measured. The abhymenial hairs measured 46–85.5 × 5.5–9 µm (n = 10) and were almost consistent with the original description (30–80 × 5–7 µm), and the basidia were 35.5–42.5 × 4–7 µm (n = 10), which is shorter than the original description (ca. 70 × 5 µm)³⁾.

Specimens previously considered as Auricularia polytricha

The nine specimens previously identified as *A. polytricha* were all considered to be different species. Of these, three specimens were reidentified as *A. cornea* as described above. This result supports the conclusion of Shirouzu et al. that *A. cornea* is included among the specimens previously identified as *A. polytricha* in Japan¹⁵⁾. The remaining six specimens (TNS-F-6569, 6574, 6609, 6610, 6650, and 195396) all had abhymenial hairs less than 300 μ m long, shorter than those of *A. polytricha* and *A. nigricans*, which are known to have abhymenial hairs longer than 600 μ m^{3, 5)}. TNS-F-6574, 6609, 6610, 6650, and 195396 could not be identified at the species level because the specimens were in poor condition, and sufficient numbers of basidiospores could not be measured. For TNS-F-6569, no species with corresponding morphological

characteristics were found among the previously known species.

TNS-F-193009 and 6563 are the type specimens of *A. polytricha* f. *leucochroma* and *A. polytricha* f. *tenuis*, respectively. Both specimens exhibited abhymenial hairs less than 400 µm long and were therefore not identified as *A. polytricha* or *A. nigricans*. The abhymenial hairs of TNS-F-193009 measured 131–375 × 6–10.5 µm (n = 10), shorter than the original description ($500-700 \times 3-8$ µm), and the basidia were $37.5-50 \times 3.5-5$ µm (n = 10), almost consistent with the original description ($45-50 \times 2.5-3.5$ µm)¹³. The abhymenial hairs of TNS-F-6563 were 84–183.5 × 5.5–7 µm (n = 10), longer than the original description ($25-125 \times 4-6$ µm), and the basidia were $37.5-53.5 \times 4.5-7$ µm (n = 10), almost consistent with the original descriptions included the morphological characteristics of their basidiospores. In this study, basidiospores were not observed in these specimens. Because of the lack of some critical characteristics, namely, basidiospores, we do not propose new combinations or names for these taxa, although they should not be placed under *A. polytricha*.

Conclusion

Species identification of 26 Auricularia specimens deposited in the TNS were morphologically reexamined, and seven of them were reidentified as different species. Reexaminations of existing specimens based on recent taxonomic criteria will contribute to updating the understanding of taxonomy and distribution of Auricularia spp. in Japan.

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Auricularia 標本の更新された形態的基準に基づく分類学的再検討

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要 旨

国立科学博物館に収蔵されている Auricularia 標本の形態を観察し,近年の分類学的基準に基づく種同定および 本属菌の日本における分類と分布の再検討を行った。その結果, A. auricula-judae とされていた 10 標本中,4 標本 がそれぞれ A. americana (1), A. cornea (1), A. villosula (2) に再同定された。残りの6 標本は,種の識別に有用 な形態的特徴がみられなかったため,種同定には至らなかった。Auricularia minor とされていた5 標本のうち,2 標 本は本種とは別種と考えられた。残りの2 標本とタイプ標本は状態が良好ではなく,十分な担子胞子の観察ができ なかった。Auricularia polytricha とされていた9 標本のうち,3 標本はA. cornea に再同定された。残りの6 標本はA. polytricha や A. nigricans とは別種であると考えられた。Auricularia polytricha f. leucochroma と A. polytricha f. tenuis のタ イプ標本は状態が良好ではなく,担子胞子の観察ができなかった。近年の分類基準に基づいて過去の標本を再検 討することにより,日本における Auricularia spp. の分類と分布情報を更新することができるだろう。