

Pigments and new lichen substances in the lichen genus *Dirinaria*

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Abstract

The new combination *Dirinaria endocrocea* (D.D.Awasthi) Kalb, Schumm & Elix is proposed for *Dirinaria confusa* var. *endocrocea* D.D.Awasthi, and the new name *Dirinaria rhodocladonica* Kalb, Schumm & Elix is proposed for *Dirinaria confluens* var. *coccinea* (Lyngé) D.D.Awasthi. Melanoclinin A & B, two pigments of unknown structure, were found in the apothecial pruina of *Dirinaria melanoclina*, *D. pruinosa* and *D. purpurascens*. The anthraquinone diacetylgraciliformin was identified in the lower medulla of most *Dirinaria* species. The naphthaquinones canarione and rhodocladonic acid were detected in the medulla of *Dirinaria leopoldii* and *D. coccinea*, and the latter substance was also found in the medulla of *D. endocrocea* and *D. rhodocladonica*. The relative Rf values for the pigments are recorded, and a key is provided to the *Dirinaria* species treated in this paper.

Introduction

Prior to the publication of "Some Lichens from tropical Africa. V" (Dodge 1971) and the world monograph of *Dirinaria* (Awasthi 1975), various species of *Dirinaria* were included in the genus *Pyxine* as *Pyxine* sect. *Dirinaria* (Tuckerman 1877) or in *Physcia* either as *Physcia* sect. *Dirinaria* (e.g. Vainio 1890; Zahlbruckner 1907, 1926, 1931) or as *Physcia* subgen. *Hypomelaena* (e.g. Lyngé 1925; Thomson 1963). Although several species are difficult to distinguish (e.g. *Dirinaria picta* (Sw.) Schaer. ex Clem. and *D. applanata* (Fée) D.D.Awasthi or *D. confluens* (Fr.) D.D.Awasthi and *D. subconfluens* D.D.Awasthi), others are readily differentiated by the orange-red or brownish red pigments present in their thalline medulla or as a pruina on their apothecial discs. In this paper, we report on our efforts to identify those pigments.

Material and methods

Thin-layer chromatographic (TLC) studies were performed in solvents A, B' and C (Elix 2014). To confirm the identity of the pigments, co-chromatograms with authentic samples of rhodocladonic acid and canarione (from an extract of *Lethariella canariensis*) were performed.

Results

Apart from several species with a pale lower cortex (*viz.* *D. complicata* D.D.Awasthi and *D. melanocarpa* (Müll.Arg.) C.W.Dodge, all the *Dirinaria* species examined produce an ochraceous, K+ purple pigment in the lower part of the medulla (Figures 1 and 2). However, it often eludes TLC investigations because in most specimens it is present in low concentration and is not evenly distributed in the thallus. To determine its TLC properties, we used a specimen of *Dirinaria aegialita* (Ach.) B.J.Moore (Schumm 20315) with the pigment present at the lobe apices in relatively high concentration (Figure 3). It produced a yellow spot on

the TLC plates with relative Rf values 38, 34, 24, and was identified as the anthraquinone diacetylgraciliformin, first described from *Cladonia graciliformis* Zahlbr. (Ejiri *et al.* 1975).

Three *Dirinaria* species are known to have a reddish, reddish brown or purplish brown pruina on their apothecial discs, *viz.* *D. melanoclina* (C.Knight) D.D.Awasthi (Figure 4), *D. pruinosa* Kalb (Figure 5) and *D. purpurascens* (Vain.) B.J.Moore (Figure 6), while other species have non-pruinose or greyish-pruinose discs. In TLC the pruina produced two bluish grey spots on the plates after treatment with sulfuric acid and charring, spots that in daylight are almost invisible. We have called them melanoclinin A (Rf values: 33, 19, 28 in solvents A, B' and C) and melanoclinin B (Rf values: 52, 31, 37). On addition of 10% KOH to a section of an apothecium seen under a microscope, the pruina turns greenish yellow.

Dirinaria species with an orange-red to coccineous pigment in the upper medulla

Dirinaria coccinea (Müll.Arg.) D.D.Awasthi, *Bibliotheca Lichenologica* 2, 53 (1975)
Physcia picta var. *coccinea* Müll.Arg., *Flora* 68, 503 (1885).

Type: Africa. Kenya, Tchamtei in Duruma, Jan. 1877, J.M.Hildebrandt 2350, i.1877 (M – lectotype!, designated by D.D.Awasthi 1975: 53).

Chemistry: Atranorin (submajor), sekikaic acid (major), 4'-O-demethylsekikaic acid (minor), rhodocladonic acid (minor), canarione (submajor), terpenes.

Dirinaria endocrocea (D.D.Awasthi) Kalb, Schumm & Elix, comb. nov.
Mycobank No. MB 831496

Basionym: *Dirinaria confusa* var. *endocrocea* D.D.Awasthi, *Bibliotheca Lichenologica* 2, 60 (1975).

Type: Brazil. Rio de Janeiro, Boa Vista (in horto), G.O.A. Malme 105, 18.vii.1892 (S – holotype!; UPS – isotype!).

Chemistry: Atranorin (submajor), sekikaic acid (major), 4'-O-demethylsekikaic acid (minor), 3-hydroxynordivarinic acid (major; Rf values: 5, 3, 5), rhodocladonic acid (submajor), terpenes.

Remarks

As in other genera of the Physciaceae (e.g. *Hyperphyscia* [Moberg 1987] and *Phaeophyscia* [Moberg 1995]), the presence or absence of the red anthraquinone skyrin is used to delimit taxa at the species level. The species *Hyperphyscia pandani* (H.Magn.) Moberg, *Phaeophyscia endococcinea* (Körg.) Moberg, *P. pyrrophora* (Poelt) Awasthi & Joshi and *P. rubropulchra* (Degel.) Essl. are all distinguished from other members of the two genera by their orange to red medulla due to the presence of skyrin. With *Dirinaria*, we are similarly using the presence or absence of the red naphthaquinone rhodocladonic acid as a diagnostic character at the species level. Thus, in our opinion the presence of rhodocladonic acid in *Dirinaria confusa* var. *endocrocea* warrants its recognition as a separate species (a description of it is given by Awasthi (1975)).

Dirinaria leopoldii (Stein) D.D.Awasthi, *Bibliotheca Lichenologica* 2, 89 (1975) Figure 7
Crocynia leopoldii Stein, *Jahresberichte der Schlesischen Gesellschaft für vaterländische Cultur* 66, 140 (1888).

Type: Democratic Republic of the Congo [Belgisch Congo]. Vivi, an *Ficus*-Ästen, *Ledien s.n.*, 1885/86 (G – lectotype, designated by D.D.Awasthi 1975: 89, not seen).

Chemistry: Atranorin (submajor), sekikaic acid (major), 4'-*O*-demethylsekikaic acid (minor), rhodocladonic acid (major), canarione (submajor), terpenes.

Remarks

Our TLC results confirm that the type specimens of *Dirinaria leopoldii* and *D. coccinea* have identical chemistry, and therefore the two species can be regarded as a species pair (Poelt 1970). Whereas the primary species (*D. coccinea*) is known from Africa only, its sorediate counterpart is known from south-eastern U.S.A., South America, Cuba and Africa. Two photographs showing the distribution of pigments in its thallus are presented in Schumm & Elix (2014: 277), and a full description is given in Awasthi (1975).

Dirinaria rhodocladonica Kalb, Schumm & Elix, nom. nov.

Figure 8

Mycobank No. **MB 831497**

Basionym: *Physcia aegialita* f. *coccinea* Lyngé, *Videnskapsselskapets Skrifter I. Mat.-Naturv. Klasse* 1924, **16**, 43 (1925).

Synonym: *Dirinaria confluens* var. *coccinea* (Lyngé) D.D.Awasthi, *Bibliotheca Lichenologica* **2**, 31 (1975).

Type: Brazil. Mato Grosso, Corumbá ad *Cereum arborescentem* [*Cereus* sp.], *G.O.A. Malme*, 26.vii.1894 (S – lectotype!, selected by D.D.Awasthi 1975: 31; LD, UPS – isolectotypes, not seen); Mato Grosso, Corumbá, in silva clara regionis calcariae, *G.O.A. Malme*, 8.viii.1894 (H – paratype!).

Chemistry: Atranorin (major), divaricatic acid (major), nordivaricatic acid (minor to trace), rhodocladonic acid (submajor to minor), terpenes.

Remarks

Here we propose raising *Dirinaria confluens* var. *coccinea* to species level using the same argument that we did with *Dirinaria endocrocea*. However, we could not use the epithet *coccinea*, because *D. coccinea* (Müll.Arg.) D.D.Awasthi already exists.

The epithet is derived from the chemistry of the species. A description is given in Awasthi (1975, as *Dirinaria confluens* var. *coccinea*).

Key to *Dirinaria* species with reddish brown, purplish brown, red-purple or coccineous pigments

- 1 Pigments restricted to the discs of the apothecia; divaricatic acid present2
1: Pigments located in the medulla of the thallus; divaricatic or sekikaic acid present4

- 2 Thallus without vegetative propagules; North and South America, South Africa, Australia*Dirinaria purpurascens*
2: Thallus with soralia or polysidiangia3

- 3 Thallus with soralia; South Africa, Australia*Dirinaria melanoclina*
3: Thallus with polysidiangia; South America*Dirinaria pruinosa*

- 4 Thallus with soralia; sekikaic acid present; south-eastern U.S.A., South America, Cuba and Africa*Dirinaria leopoldii*
4: Thallus without vegetative propagules; divaricatic or sekikaic acid present5

- 5 Divaricatic acid present; South America*Dirinaria rhodocladonica*
5: Sekikaic acid present, South America or Africa6

- 6 Medulla with rhodocladonic acid and canarione; Africa*Dirinaria coccinea*
6: Medulla with rhodocladonic acid only; South America*Dirinaria endocrocea*

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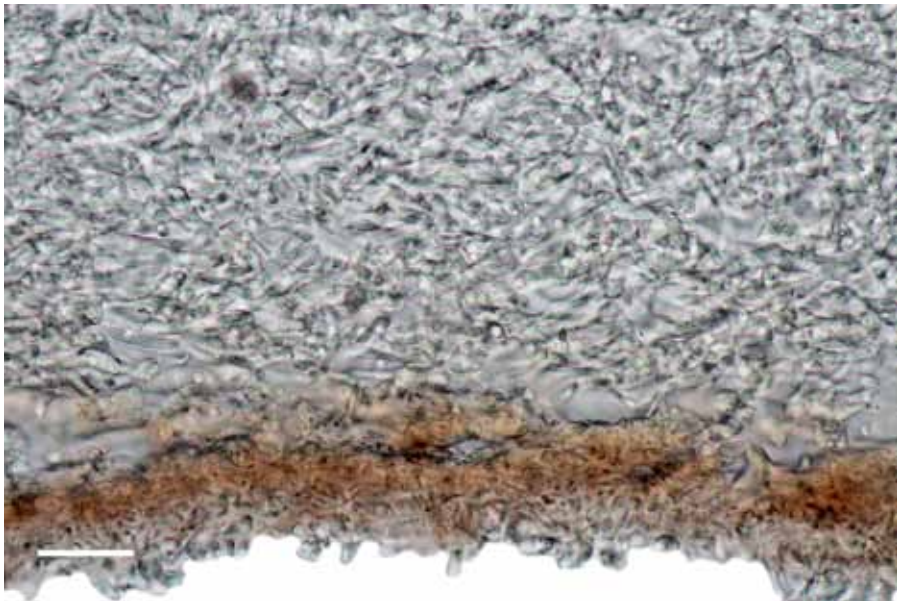


Figure 1. *Dirinaria aegialita* (Schumm 20315 – herb. F. Schumm); longitudinal section through lower part near the lobe apex, showing the ochraceous layer with the anthraquinone diacetylgraciliformin. Bar = 20 μ m.



Figure 2. *Dirinaria aegialita* (Schumm 20315 – herb. F. Schumm); lower side of lobe apices, showing the K⁺ purple reaction (arrow) of diacetylgraciliformin. Bar = 0.5 mm.



Figure 3. *Dirinaria aegialita* (Schumm 20315 – herb. F. Schumm); lower side of lobe apices, showing the high concentration of the ochraceous pigment diacetylgraciliformin and the hapters. Bar = 1 mm.



Figure 4. *Dirinaria melanoclina* (Kalb 33905 – herb. K.Kalb). Apothecia, showing the thick purplish brown pruina with melanoclinin A and melanoclinin B. Bar = 0.5 mm.



Figure 5. *Dirinaria pruinosa* (Kalb 26837 – herb. K.Kalb). Apothecia, showing the granular purplish brown pruina with melanoclinin A and melanoclinin B. Bar = 0.5 mm.



Figure 6. *Dirinaria purpurascens* (Kalb 21119 – herb. K.Kalb), showing the thin purplish-brown apothecial disc pruina with melanoclinin A and melanoclinin B. Bar = 0.5 mm.



Figure 7. *Dirinaria leopoldii* (Kalb 11346 – herb. K.Kalb). Subdichotomously or pinnately divided lobes with flabellate apices and cinnabar-red coloured soralia, caused by a mixture of rhodocladonic acid and canarione. Bar = 2 mm.

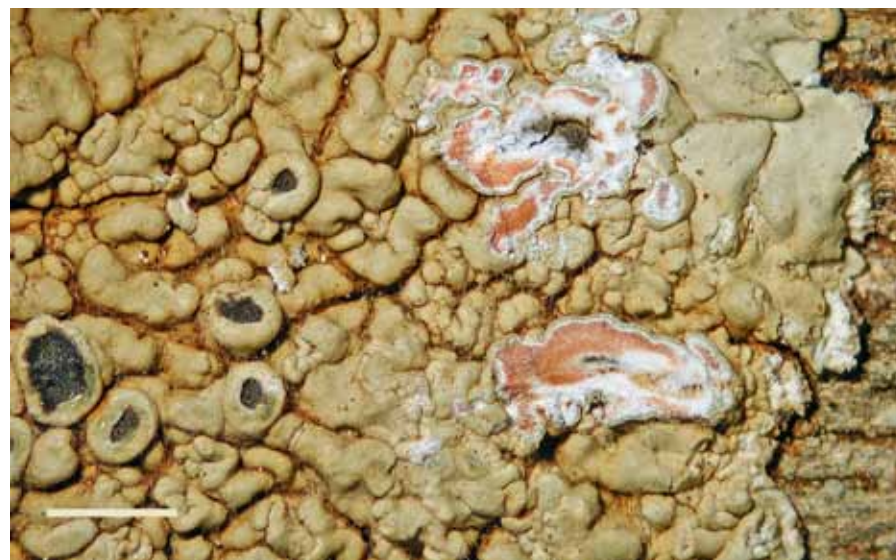


Figure 8. *Dirinaria rhodocladonica* (Kalb 42542 – herb. K.Kalb). Upper part of the red-purple medulla caused by rhodocladonic acid and seen when the cortex is removed. Bar = 1 mm.